NEW ASPECTS ON STABILITY OF SOME EDIBLE OILS

By

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ABSTRACT

Rania Ibrahim Mohammad Almoselhy: New Aspects on Stability of some Edible Oils. Unpublished Ph.D Thesis, Department of Food Science, Faculty of Agriculture, Ain Shams University, 2015.

The present study was planned to evaluate the stability of four edible oils namely extra virgin olive oil (EVOO), moringa oil (MO), apricot kernel oil (AKO) and sunflower oil (SO). The oils were analyzed in order to identify the relation between their composition and stability, with great stress to investigate what components are responsible for their stability.

Fourier transform infra red (FTIR) and nuclear magnetic resonance (NMR) spectroscopic determinations were also applied. MO showed the highest induction period (IP) measured by Rancimat being 190.00 hr indicating superior resistance to oxidation, followed by EVOO (59.11 hr), then AKO (26.00 hr). SO recorded only 7.45 hr. The highest resistance of MO to oxidation followed by EVOO was due to their less linoleate (LO), higher percentage of oleate (OL) and saponifiable matter, as well as antioxidant content of tocopherols, flavonoids, sterols, phenolics, chlorophyll, and carotenoids. In addition, MO contained the highest percentages of stigmasterol, campesterol, Δ^5-avenasterol and δ-tocopherol which its antioxidant activity exceeds that of α- and γ-tocopherols and making clear the long IP of this oil. The highest percentages of β-sitosterol was found in EVOO, besides Δ^5-avenasterol that may be mainly responsible for olive oil stability, also polyphenols were the main antioxidants in EVOO that approached half of its IP.

AKO with middle IP had relatively high stability due to its higher percentage of OL, but it had higher LO and lower antioxidant content compared to MO and EVOO. The lowest IP of SO was due to its higher PUFA and lower antioxidant content. It seems that β-sitosterol and Δ^5-avenasterol were also a main factor in AKO and SO oxidative stability.

From FTIR spectral data of the tested oils, the shift of absorption peak around 3005 cm^{-1} ordered the oils, where the lowest shift was related to the lowest PUFA and the highest stability which arranged as follows:

MO (3003.59 cm^{-1}) > EVOO (3004.55 cm^{-1}) > AKO (3005.52 cm^{-1}) > SO (3006.48 cm^{-1}).

This arrangement reflects the same order of oils stability evaluated by Rancimat method. Also, the higher in intensity of this peak around 3005 cm^{-1}, the higher in USFA From this relation, it was easy to predict the order and the approximate values of the USFA in the oils. When the oil shows a peak around 3470cm^{-1}, it is an indication that the oil is non-oxidized.

The chemical shifts of solvent peaks around 2.5 and 3.3 ppm in the ^{1}H-NMR spectra ordered the oils stability, where the lesser shift was corresponded to the oil of higher stability, this order was as obtained by Rancimat; it also agreed with the order of tocopherols content. MO showed the presence of both stigmasterol and β-sitosterol from the signals at 0.662 and 0.784 ppm, respectively. However, EVOO showed a peak at 0.780 ppm assigned to β-sitosterol. Some shifts were denoted in the spectra of the stored oils for 6 months attributed to free fatty acids indicating hydrolytic degradation of acyl groups and the formation of new compounds, as well as conjugated systems. Also, new signals were aroused after 60 months due to different kinds of alcohols, free fatty acids and diglycerides.

All the ^{13}C-NMR spectral data from the signals in the four regions of the tested oils, agreed with their GC and the other composition analysis carried out, and reflect the same composition and order of oils stability obtained by Rancimat method. In addition, its technique is particularly useful in distinguishing between mono-, di-, and triglycerides.

In conclusion, FTIR and NMR spectroscopy proved their potency as new analytical methods to investigate the stability of some edible oils and the results obtained agreed with the old common methods of analyses.

Key Words:

Stability; ^{1}H-NMR spectroscopy; ^{13}C-NMR spectroscopy; FTIR spectroscopy; UV spectroscopy; GC analysis; HPLC analysis; extra virgin olive oil; moringa oil; apricot kernel oil; sunflower oil.

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LIST OF ABBREVIATIONS

ACN/THF	Acetonitrile/Tetrahydrofuran
AKO	Apricot kernel oil
ALA	Alpha linolenic acid
An-V	Anisidine value
AOCS	American Oil Chemists' Society
AOM	Active Oxygen Method
AP	Ascorbyl palmitate
ASTM	American Society for Testing and Materials
ATR-FTIR	Attenuated Total Reflectance-Fourier Transform Infra Red
AV	Acid value
BHT	Butylated hydroxyl toluene
BP	Boiling Point
$CDCl_3$	Deuterated chloroform
CL	Chemiluminescence
COPs	Conjugable oxidation products
CPMSO	Cold pressed moringa seed oil
DAG	Diacyl glycerol
DG	Diglyceride
DHA	Docosa hexaenoic acid
DMSO-d6	Deuterated dimethyl sulphoxide
DNPH	Dinitrophenylhydrazine
DPPH	1,1-Diphenyl-1-picryl hydrazyl
DSC	Differential Scanning Calorimetry
EDTA	Ethylene diamine tetra acetic Acid
ELISA	Enzyme-Linked Immunosorbent Assay
EPR	Electron Paramagnetic Resonance
ESR	Electron Spin Resonance
EVOO	Extra virgin olive oil
FA	Fatty acid
FFA	Free fatty acid

FID	Flame Ionization Detector
FIR	Far-IR
FOX	Ferrous oxidation-xylenol orange
FTIR	Fourier Transform Infra Red
FT NIR	Fourier Transform Near Infra Red
GC	Gas Chromatography
GC–FID	Gas Chromatography – Flame Ionization Detector
GC-MS	Gas Chromatography–Mass Spectrometry
GLC	Gas Liquid Chromatography
HEMSO	Hexane extracted moringa seed oil
HOSO	High oleic sunflower oil
HPLC	High-Performance Liquid Chromatography
IOC	International Olive Council
IP	Induction period
IV	Iodine value
LN	Linolenic
LO	Linoleic
LOD	Limit of detection
MA	Malonaldehyde
MAG	Monoacyl glycerol
MIR	Mid-infrared
MO	Moringa oil
MRI	Magnetic Resonance Imaging
MS/FID	Mass Spectroscopy / Flame Ionization Detector
MUFA	Monounsaturated fatty acid
NIR	Near-infrared
NMR	Nuclear Magnetic Resonance
OL	Oleic
OOT	Onset oxidation temperature
OSET	Oxidative stability at elevated temperatures
OSI	Oil stability index
Ox-OO	Oxidized olive oil

O/W	Oil in water
PBN	α-phenyl tert-butyl nitrone
PCA	Principal component analysis
PDSC	Pressure Differential Scanning Calorimetry
PUFA	Polyunsaturated fatty acid
PV	Peroxide value
Rad	Ratios of aliphatic to diallylmethylene protons
Rao	Ratios of aliphatic to olefinic protons
RP-HPLC	Reversed Phase – High Performance Liquid Chromatography
RSA	Radical scavenging activity
Sat	Saturates
SD	Standard deviation
SFA	Saturated fatty acid
SO	Sunflower oil
TAG	Triacylglycerol
TBA	Thiobarbituric acid
TBARS	Thiobarbituric acid reactive substances
TBHQ	Tert-butyl hydroquinone
TF	Total flavonoids
TG	Triglyceride
THF	Tetrahydrofuran
TMS	Tetra methyl silane
TPM	Total polar matter
TPP	Triphenylphosphine
TPPO	Triphenylphosphine oxide
USFA	Unsaturated fatty acids
UV	Ultra violet
VOO	Virgin olive oil
w/v	weight/volume

1-INTRODUCTION

Dietary lipids, naturally occurring in raw food materials or added during food processing, play an important role in food nutrition and flavor. Meanwhile, lipid oxidation is a major cause of food quality deterioration, and has been a challenge for manufacturers and food scientists alike. Lipids are susceptible to oxidative processes in the presence of catalytic systems such as light, heat, enzymes, metals, metalloproteins, and micro-organisms, giving rise to the development of off-flavors and loss of essential amino acids, fat-soluble vitamins, and other bioactives.

Lipids may undergo autoxidation, photo-oxidation, thermal oxidation, and enzymatic oxidation under different conditions, most of which involve free radical or oxygen species. Autoxidation is the most common process leading to oxidative deterioration and is defined as the spontaneous reaction of atmospheric oxygen with lipids.

Unsaturated fatty acids are generally the reactants affected by such reactions, whether they are present as free fatty acids, triacylglycerols (as well as diacyglycerols or monoacylglycerols), or phospholipids. Autoxidation of unsaturated fatty acids occurs via a free radical chain reaction that proceeds through three steps of initiation, propagation, and termination. As oxidation normally proceeds very slowly at the initial stage, the time to reach a sudden increase in oxidation rate is referred to as the induction period.

Lipid hydroperoxides have been identified as primary products of autoxidation; decomposition of hydroperoxides yields aldehydes, ketones, alcohols, hydrocarbons, volatile organic acids, and epoxy compounds, known as secondary oxidation products. These compounds, together with free radicals, constitute the bases for measurement of oxidative deterioration of food lipids. Numerous analytical methods are routinely used for measuring lipid oxidation in foods. However, there is no standard method for detecting all oxidative changes in all food systems. Therefore, it is necessary to select a proper and adequate method for a particular application **(Shahidi and Zhong, 2005).**

There is a considerable interest in polyphenols and carotenoids because of their antioxidant properties and ability to alleviate chronic diseases. The carotenoids are the most widespread group of pigments in nature, and they are present in all photosynthetic organisms and are responsible for most of yellow to red colors of fruits, flowers and seed oils. Ripening of the fruits involves series of complex biochemical reactions which lead to production of phenolic compounds, carotenoids and the formation of volatile compounds. The differences in contents and quantities of previously mentioned phytochemicals may occur and depends on a number factors, sunlight, soils, season, region of cultivation, fruit and seed variety, stages of maturity **(Dragovic-Uzelac *et al.*, 2007).**

Polyphenols are synthesized by plants as a response to environmental stress and microbial infections, and are known to have antioxidant, anti-inflammatory and antimicrobial properties. Numerous studies have shown that polyphenols are important preventive agents against several degenerative diseases and different types of cancer such as breast, prostate, skin, and colon cancer. The contribution of phenolic components to the shelf life and to the oxidative stability of olive oil has also been established **(Anastasopoulos *et al.*, 2012).**

Phytosterols are minor components of vegetable oils and form a major proportion of the unsaponifiables. Phytosterols in vegetable oils are important from a nutritional point of view because they contribute to lowering serum cholesterol levels in humans and to oxidative and thermal stability and shelf-life **(Azadmard-Damirchi and Dutta, 2008).**

The oxidative stability is an important indicator for the quality of oil. There are various methods available for measurement of lipid oxidation in oil foods. Up to date, the available methods to monitor lipid oxidation in foods are based on chemical analysis involving several reagents and sample preparation steps, which may be divided into two groups: primary and secondary oxidative changes. The peroxide value (PV) is normally used as the method for quantitative of hydroperoxides, which are measured to determine the primary oxidative change because they are generally accepted as the first product formed by oxidation. The anisidine value (AnV) is utilized to determine the secondary oxidative products, which are derived from decomposition of hydroperoxides. Such methods are rather costly and time-consuming, and therefore, an alternative rapid and reliable method should be considered.

Fourier transform infrared spectroscopy (FTIR) is an analytical technique, which measures the infrared intensity versus wavenumber of light. The resulting spectrum is characteristic of the organic molecules, which absorb infrared energy at specific frequencies so that the basic structure of compounds can be determined by the spectral locations of their IR absorptions. Numerous reports have shown that the FTIR has been applied to evaluate the freshness of virgin olive oils in combination with multivariate analysis, to determine fatty acid profile and PV of virgin olive oil, to analyze the free fatty acid content of Atlantic salmon (*Salmo salar*) skin lipids, also to determine *cis*-unsaturation and *trans*-fatty acids together with free fatty acids during degradation of edible oils by heating process. FTIR can be used to investigate lipid oxidation of oil and many other applications as mentioned by **Klaypradit *et al.* (2011).**

Infrared (IR) spectroscopy is a very helpful way to study lipid degradation under oxidative conditions, particularly since it is an easy, rapid, economical and non-destructive technology. It is based on the determination of fundamental vibrational transitions of a particular compound and involves the absorption of discrete energy levels from the IR region. These discrete energy levels are characteristic of each atom–atom linkage, so studying the IR spectrum can provide enough information to find out the nature of the analyzed compound. Differences were observed in the IR spectra of fresh and aged oils. IR spectra could characterize the ageing of various edible oils **(Barriuso *et al.*, 2013).**

Edible oils are mainly made up of triacylglycerols (TAG) which comprise more than 95 to 99% of the total lipids present. In TAG the HO- of the glycerol joins the -COOH of the fatty acid (FA) to form ester bonds. Because there are a large number of individual FAs, with different chain lengths, and degrees of unsaturation and position on the glycerol molecules, defining the TAG composition of an oil is a very challenging task. In fact, each type of oil has a different TAG profile which determines the nature of its physicochemical and nutritional properties, and also provides information on the quality of the oil. Several methods for the qualitative and quantitative determination of TAGs in oil samples are available. These techniques include mainly gas-liquid chromatography, high performance chromatography in normal and reversed phase mode, thin-layer chromatography and supercritical fluid chromatography. However, these methods are labor-intensive and time consuming and also involve a complex series of chemical manipulation steps. Nuclear magnetic resonance (NMR) spectroscopy has become one of the most promising methods to determine organic structures in complex matrices such as foods, and pharmaceutical and biological samples. It has a long history as an academic research tool, and is a cornerstone of modern analytical chemistry in laboratories across the world**.** ^{1}H nuclear magnetic resonance (^{1}H-NMR) offers many advantages over alternative analytical methods to study edible oils because it allows the rapid, simultaneous, noninvasive, and nondestructive study of oil composition, and also provides information about the acyl distribution and the acyl positional distribution of TAGs **(Salinero *et al.*, 2012; Parker *et al.*, 2014).**

Low resolution NMR has been used for a long time, to determine the solid fat content in a sample, the melting point curves of semi-solid fats, or the percentage by weight of oil in foods. High resolution ^{1}H and ^{13}C-NMR have also been used for a long time in the study of food lipids. ^{1}H-NMR is related to the energy levels of ^{1}H nuclei that result when a sample is placed within an external magnetic field. Each ^1H nucleus in each molecule is surrounded by electrons and other nuclei which cause small local modifications to the external field. Nuclei of hydrogen atoms in different chemical environments have slightly different separations of their energy levels. These energy level separations may be measured very accurately as frequencies using the Fourier transform NMR technique, which monitors the response of the nuclei after they have been perturbed from equilibrium by a short intense pulse of radio-frequency electromagnetic radiation. The NMR spectrum is a series of sharp signals whose frequencies can be related to the chemical nature of the hydrogen atoms (methyl, methylene groups, etc.) and whose intensities are directly related to the number of hydrogens producing the signal **(Guillén and Ruiz, 2001).**

High-resolution NMR plays an important role in oil analysis. This is firstly due to the absence of sample pretreatment, since oils are simply added to a solvent before analysis. ^1H NMR studies of oils have increased because of the great amount of information that high field NMR instruments can provide with a single experiment and in a very short time. This entry is mainly focused on the qualitative and quantitative information that can be obtained from the oils ^1H NMR spectra regarding major and minor components **(Brescia and Sacco, 2006).**

AIM OF INVESTIGATION

The objective of the present study was to develop reliable new methods for the evaluation of stability of some edible oils, other than the common methods which are labor-intensive, time consuming and involve a complex series of chemical manipulation steps. Also, to investigate which components in some edible oils such as EVOO, MO and AKO caused their high stability comparing them with those in some edible oils of low stability (SO) to deduce the components responsible for the high stability.

These objectives were achieved throughout the following fulfill:

- Preparing fresh and oxidized samples of EVOO, MO, AKO and SO to be investigated by manual and instrumental methods of analyses. The applicable analytical determinations were as follows:

1- Evaluation of some physicochemical quality parameters as: sensory evaluation, color, refractive index, acidity, peroxide value, iodine value, UV spectroscopy at 232, 270, 266, 274 nm, ΔK, carotenoids, chlorophyll, total unsaponifiable matters and saponification number.
2- Application of GC for the identification of fatty acids.
3- Application of HPLC for the identification of phenols, flavonoids and tocopherols.
4- Evaluation of oxidative stability by the Rancimat method.
5- Evaluation of antioxidant activity by DPPH method.
6- Application of FTIR spectroscopy to explore its capabilities in the evaluation of oils stability.
7- Application of NMR spectroscopy (^{1}H, ^{13}C) to explore its capabilities in the evaluation of oils stability.

- Deducing and developing the useful analytical assay which accurately reflects the stability of the edible oils, as well as which components are responsible for the high stability.

2- REVIEW OF LITERATURE

Several physical and chemical parameters have been proposed by several investigators for some edible oils as following:

2.1- Physicochemical Quality Parameters of Edible Oils

Quality characteristics of extra-virgin olive oils depend on several factors. **Baiano *et al.* (2013)** studied the effects of genotype and growing location on olive oil quality, olives from cv. Coratina, Nocellara, Ogliarola, and Peranzana, picked in four locations of the Apulia region (Italy), were crushed by a three-phase system to produce mono-cultivar extra virgin olive oils and analyzed for their acidity, peroxide value, spectrophotometric indices, total phenolic content, phenolic profile and antioxidant activity. The experimental data concerning peroxide value, spectrophotometric indices, phenolic content and profile and antioxidant activity showed great variability among the cultivars grown in the same location and also among the oils produced with olives of the same cultivar but grown in different locations. For each cultivar, no significant differences were found among locations in terms of acidity and ΔK whereas peroxide value, K_{232}, and K_{270} differ significantly among locations for both Ogliarola and Peranzana cv. Concerning the phenolic content of Ogliarola cv., no differences were highlighted between the locations whereas the phenolic contents of Peranzana significantly changed as a function of the place of growing. On the basis of these results, the statistical multivariate analysis did not allow the classification into homogeneous groups neither of the oils belonging to the same cultivar nor of those obtained from olives picked in the same location.

The regulated physicochemical and sensory parameters, stability parameters and fatty acid, sterol and triterpenic dialcohol composition of the olive oils from the varieties Arbequina, Benizal, Cornicabra, Cuquillo, Injerta, Manzanilla de Sevilla, Manzanilla Local, Picual and Negrilla, grown in the Campos de Hellin, were analyzed by **Pardo *et al.* (2013).**

Regarding potential quality, all the oil samples were classified in the ‘‘extra virgin’’ category according to the regulated parameters. The oils from the varieties Cornicabra and Picual showed remarkably high stability, due to their high total phenol content. The oils from the Benizal variety stood out due to their high campesterol and low total sterol content, exceeding and not reaching, respectively, the limits set by European regulations. This seems to be an intrinsic characteristic of this variety. When the real quality was analyzed, two clearly differentiated groups were observed: on one hand, oils from the Arbequina variety, and on the other hand, oils from the Picual variety and oils mixed from different varieties (blends). The great number of olive varieties grown in the Campos de Hellin area enables the production of better balanced oils, producing high quality blended oils since the mixing of different varieties may compensate the deficiencies of monovarietal oils.

Dabbou *et al.* (2010) studied the quality indices of virgin olive oils from various varieties grown in Tunisia. The physical and chemical parameters of 5 cultivars; *Arbequina*, *Koroneiki*, *Leccino, Oueslati, Chemchali* were as follows: FFA (% of oleic acid), 0.20, 0.62, 0.48, 0.27, 0.24; peroxide value (meq O^2/kg), 6.67, 5.92, 3.92, 11.50, 8.84; iodine value (g I_2/100 g oil), 89.67, 85.13, 81.98, 92.97, 94.40; *p*-AV, 2.23, 2.08, 1.81, 2.29, 2.95; Totox value, 15.56, 13.90, 9.64, 25.29, 20.62; K_{232}, 2.47, 1.81, 1.89, 2.46, 2.33; K_{270}, 0.21, 0.22, 0.19, 0.21, 0.21, respectively.

Anwar *et al.* (2005) examined the interprovenance variation in the composition of *Moringa oleifera* oilseeds from Pakistan. The hexane-extracted oil content of *Moringa oleifera* seeds harvested in the vicinity of the University of Agriculture, Faisalabad (Punjab, Pakistan), Bahauddin Zakariya University (Multan, Pakistan), and the University of Sindh, Jamshoro (Sindh, Pakistan), ranged from 33.23 to 40.90%. Protein, fiber, moisture, and ash contents were found to be 28.52–34.00, 6.52–7.50, 5.90–7.00, and 6.52–7.50%, respectively. The physical and chemical parameters of the extracted *Moringa oleifera* oils were as follows: iodine value, 67.20–71.00; refractive index (40°C), 1.4570–1.4637; density (24°C), 0.9012–0.9052 mg/mL; saponification value, 177.29–184.10; unsaponifiable matter, 0.60–0.83%; color (1-in. cell), 1.00–1.50R + 20.00–30.00Y; smoke point, 198–202°C; and acidity (% as oleic acid), 0.50–0.74. Tocopherols (α, γ, and δ) accounted for 114.50–140.42, 58.05–86.70, and 54.20–75.16 mg/kg, respectively, of the oils. The induction periods (Rancimat, 20 L/h, 120°C) of the crude oils were 9.64–10.66 h and were reduced to 8.29–9.10 h after degumming. Specific extinctions at 232 and 270 nm were 1.80–2.50 and 0.54–1.00, respectively. The major sterol fractions of the oils were campesterol (14.13–17.00%), stigmasterol (15.88–19.00%), β-sitosterol (45.30–53.20%), and Δ^5-avenasterol (8.84, 11.05%). The *Moringa* oils were found to contain high levels of oleic acid (up to 76.00%), followed by palmitic, stearic, behenic, and arachidic acids up to levels of 6.54, 6.00, 7.00, and 4.00%, respectively. Most of the parameters of *Moringa oleifera* oils indigenous to different agroclimatic regions of Pakistan were comparable to those of typical *Moringa* seed oils reported in the literature. The results of this analytical study, compared with those for different vegetable oils, showed *Moringa. oleifera* to be a potentially valuable oilseed crop.

Manzoor *et al.* (2007) carried out a study on *Moringa concanensis.* They reported the physicochemical characterization of *Moringa concanensis* seed oil from Tharparkar (a drought hit area), Pakistan. The hexane-extracted oil content of *Moringa concanensis* seeds ranged from 37.56 to 40.06% (average 38.82%). The extracted oil exhibited an iodine value of 67.00; a refractive index (40^{o}C) of 1.4648; its density (24^{o}C) was 0.8660 mg mL^{-1}; the saponification value (mg of KOH g^{-1} of oil) was 179.00; unsaponifiable matter 0.78%; color (1 in. cell) 1.90R + 19.00Y; and acidity (% as oleic acid) 0.34%. Tocopherols (α, γ, and δ) in the oil accounted for 72.11, 9.26 and 33.87 mg kg^{-1}, respectively. Specific extinctions at 232 and 270 nm were 3.17 and 0.65, respectively. The peroxide and *p*-anisidine values of the oil were found to be 1.75 and 1.84 meq kg^{-1}, respectively. The induction periods (Rancimat, 20 L h^{-1}, 120^{o}C) of the crude oil was 10.81 h and reduced to 8.90 h after degumming. The *Moringa concanensis* oil was found to contain high levels of oleic acid (up to 68.00%) followed by palmitic, stearic, behenic, and arachidic acids up to levels of 11.04, 3.58, 3.44 and 7.09%, respectively. These results, compared with those for other *Moringa* species and different vegetable oils, showed *Moringa concanensis* to be a potentially valuable nonconventional seed crop for high quality oil.

In this respect, **Ogunsina *et al.* (2014)** stated the physicochemical characteristics of cold pressed moringa seed oil (CPMSO) and hexane extracted moringa seed oil (HEMSO) of Indian origin as: color (Lovibond units, Y+5R) was 30.0 and 36.0, FFA (as oleic acid) % was 3.5 and 4.0, peroxide value (meq O^{2}/kg) was 1.0 and 1.02, iodine value (g I_2/100 g oil) was 67.8 and 68.5, saponification value (mg KOH/g oil) was 190.4 and 191.2, unsaponifiable matter % was 0.59 and 0.65, total tocopherols (mg/kg) was 95.5 and 90.2, total polar materials % was 3.1 and 0.0, refractive index was 1.47 and 1.47, density at 25^{o}C was 0.90 and 0.92, specific gravity (g/cm^{3}) was 0.93 and 0.90 and viscosity mPa.s was 43.8 and 43.6 for CPMSO and HEMSO, respectively.

Alpaslan and Hayta (2006) reported the values of specific gravity, refractive index, iodine, saponification number, and unsaponifiable matter for apricot kernel oil samples from a number of cultivars. The ranges were 0.1–1.6 for unsaponifiable matter, 187.3–199.0 for saponification number, 90.0–104.8 for iodine value, 0.876–0.932 for specific gravity, and 1.464–1.480 for the refractive index.

Alpaslan and Hayta (2006) studied the physical and chemical properties of apricot kernel and mentioned that the oil content of the kernels varied from 27.7 to 66.7%. The major FA contents were oleic (58.3–73.4%) and linoleic (18.8–31.7%). The contents of unsaturated FA (91.5–91.8%) and saturated FA (7.2–8.3%) were reported, as well as neutral lipids (95.7–95.2%), glycolipids (1.3–1.8%), and phospholipids (2.0%). The kernel oil contained 11.8 mg/100 g campesterol, 9.8 mg/100 g stigmasterol, and 177.0 mg/100 g sitosterol. The values of specific gravity, refractive index, iodine, saponification number, and unsaponifiable matter for apricot kernel oil were presented. Their ranges were 0.876–0.932, 1.464–1.480, 90.0–104.8, 187.3–199.0 and 0.1–1.6, respectively.

Zhang *et al.* (2011) reported the main bioactive components of AKO as fatty acids, tocopherols and phenolic compounds. Fatty acid composition of the AKO was determined by gas chromatography–mass spectrometry, and found to consist of oleic acid (66.4%), linoleic acid (25.5%), palmitic acid (4.8%), stearic acid (1.2%), palmitoleic acid (1.1%), linolenic acid (0.5%), peanut monoenoic acid (0.2%), erucic acid (0.2%) and arachic acid (0.1%). Tocopherol content was determined to be 22.0 ± 0.6 mg/100 g oil via HPLC. Total phenol content, determined using Folin–Ciocalteu method, was 0.18 ± 0.02 mg/g (gallic acid equivalent).

Volli and Purkait (2014) estimated some physical and chemical properties for sunflower oil and they found that: pH was 4.27, water content (% vol) was 0.01, specific Gravity (gmcc^{-1}) was 0.923, viscosity@40^{o}C (cP) was 33.01, acid value (mg KOH g-1) was 0.987, refractive index was 1.472. However, **Guiotto *et al.* (2014)** reported some chemical properties for sunflower oil as follows: free fatty acids (% oleic acid) was 0.06, peroxide value (mequiv O_2/kg) was 1.5, *p*-anisidine value was 5.1 and induction time (h) was 13.0.

2.2- Fatty Acid Composition of Edible Oils

Chugh and Dhawan (2014) evaluated the fatty acid composition (%) of olive oil as follows: palmitic: 14.8, oleic: 70.0, linoleic: 12.7, linolenic: 1.0, eicosenoic: 0, behenic: 0, erucic: 0, MUFA: 4.73, PUFA: 0.92.

Ogunsina *et al.* (2014) stated the fatty acid composition (%) of cold pressed moringa seed oil (CPMSO) and hexane extracted moringa seed oil (HEMSO) of Indian origin as follows: myristic was 0.24 and 0.72, palmitic was 5.8 and 6.1, palmitoleic was 1.2 and 1.2, stearic was 3.9 and 4.6, oleic was 79.5 and 78.7, linoleic was 0.0 and 0.0, linolenic was 2.2 and 1.8, arachidic was 2.2 and 2.3, gadoleic was 0.0 and 0.0, behenic was 5.1 and 4.5, % SFA was 17.2 and 18.3, % MUFA was 80.7 and 79.9, % PUFA was 2.2 and 1.8 for CPMSO and HEMSO, respectively.

Durmaz *et al.* (2010) found that oleic acid (18:1) was found to be the primary fatty acid in unroasted AKO (68.69%). Linoleic acid (18:2) and palmitic acid (16:0) contents were found to be 23.19 and 6.00% of the total fatty acids, respectively. Other fatty acids were found in relatively lower levels.

Chugh and Dhawan (2014) evaluated the fatty acid composition (%) of sunflower oil, and it was as follows: palmitic: 5.5, oleic: 34.8, linoleic: 58.60, linolenic: 0, eicosenoic: 0, behenic: 0, erucic: 0, MUFA: 6.32, PUFA: 10.65.

In this respect, **Guiotto *et al.* (2014)** found that the fatty acid composition (%) of sunflower oil was as follows: palmitic: 6.6, stearic: 2.3, oleic: 36.6, linoleic: 54.40, linolenic: 0.

2.3- Unsaponifiable Matter Composition of Edible Oils

The unsaponifiable fraction forms a minor, highly specific part of a vegetable oil, and can be used as an indicator of genuineness.

Tranchida *et al.* (2013) carried out an investigation focused on the development of a comprehensive two-dimensional GC (GC×GC) method, with dual MS/FID detection, for the qualitative and quantitative analysis of the entire unsaponifiable fraction of vegetable oils. The column set used consisted of a low-polarity first dimension, and a medium-polarity secondary one, both characterized by a high thermal stability. The use of dual detection enabled the attainment of both mass spectral information and relative % FID data. The complexity of the fingerprint, generated by the unsaponifiable fraction, fully justified the employment of the two-dimensional GC technology. Furthermore, two other GC×GC benefits contributed greatly to the attainment of promising results, namely sensitivity enhancement and the formation of group type patterns. The method herein proposed could potentially open a new opportunity for the more in-depth knowledge of the unsaponifiable fraction of vegetable oils.

2.3.1- Sterols Composition of Edible oils

The sterols are generally the major components of the unsaponifiable fractions of vegetable oils. Their structures are based on a steroidal alcohol framework comparable with that of cholesterol. The molecules are planar and are based on a tetracyclic cyclopentaphenanthrene system with four fused rings. The hydroxyl group at C-3, side chain at C-17, and two methyl groups at C-18 and C-19 are all angular to the ring structure and have β-stereochemistry. Plant sterols, or phytosterols, are generally the predominant compounds in the unsaponifiable fractions of vegetable oils that generally account for about 1% of the oils. The main sterols are belong to the 4-desmethylsterols family, while 4-methylsterols and 4,4-dimethylsterols (also called triterpene alcohols) are present as minor components in most oils. Apart from some exceptions, the desmethylsterol, β-sitosterol, is generally the most abundant and is usually accompanied by variable levels of campesterol, stigmasterol, Δ^5-avenasterol, and other sterols. Some sterols are characteristic for certain oils; e.g., brassicasterol is characteristic for rapeseed/canola oil and can be used to detect the presence of this oil in foods. Recently, it was reported that camolina oil is special because of its high content of cholesterol (188 ppm) besides brassicasterol (133 ppm), campesterol (893 ppm), stigmasterol (103 ppm), sitosterol (1884 ppm), and Δ^5-avenasterol (393 ppm). Black cumin (Nigella sativa L.) is characterized by high levels of β-sitosterol (1135–1182 ppm), Δ^5-avenasterol (925–1025 ppm), and Δ^7 avenasterol (615–809 ppm), and small amounts of stigmasterol and campesterol **(Kamal-Eldin, 2005).**

Sterols occur in vegetable oils in the free and esterified forms in relative levels that are dependent on the type of oil. In the sterol esters of vegetable oils, the hydrogen of the hydroxyl group at C-3 is substituted with a fatty acyl or with ferulic acid as in γ-oryzanols. Oils of corn, cottonseed, and rapeseed are especially rich in sterol esters. According to this report, there are no differences in the composition of the free and acylated sterol fractions, which suggests nonspecific esterification. Rice bran oil is currently recognized for its high content (about 2–5%) of γ-oryzanol, i.e., ferulic acid esters of mainly cycloartenol and 24-methylene cycloartanol, but also cyclobranol, cycloeucalenol, sitosterol, and campesterol. Two-thirds of the total sterols of wheat germ oil, 2000–4000 mg/100 g, are present in ester form. Refining affects the sterols of vegetable oils in various ways and is responsible for sterol losses in the range of 10–70%. The use of acid clay bleaching agents and elevated temperatures catalyzes different isomerization, dehydration, and esterification reactions. Bleaching effects on phytosterols are generally minor and mainly limited to the formation of some nonpolar dehydration products and partial hydrolysis of sterol esters. Deodorization, on the other hand, causes a significant reduction in the total sterol because of distillation and esterification of free sterols. Phytosterols are industrially isolated from the distillates, resulting from the deodorization of vegetable oils. Phytosterols are sometimes hydrogenated to produce phytostanols. As the solubility of sterols and stanols is very low (<1% at 25^{o}C), it limits their application in food products. Esterification of sterol and stanols is, therefore, performed to make them fat-soluble and easy to incorporate in food products **(Kamal-Eldin, 2005).**

Ben Temime *et al.* (2008) studied the sterol profile of virgin olive oils grown under different environmental conditions, by gas chromatography using a flame ionization detector. More than ten compounds were identified and characterized. As expected for virgin olive oil, the main sterols found in all Chétoui olive oils were β-sitosterol, Δ^5-avenasterol, campesterol and stigmasterol. Cholesterol, 24 methylenecholesterol, clerosterol, campestanol, sitostanol, Δ^7-stigmastenol, Δ^5,24-stigmastadienol, and Δ^7-avenasterol were also found in all samples, but in lower amounts. Most of these compounds were significantly affected by the geographical origin.

2.3.2- Tocopherols and Tocotrienols Composition

Tocopherols are natural antioxidants present in all vegetable oils. The seed oils contain four types of tocopherols at varying amounts. It is important to note the difference between the seed oil and palm oil. Seed oils contain tocopherols. Palm oil contains tocotrienols in addition to tocopherols, and also a small amount of coenzyme Q10. Both tocotrienols and coenzyme Q10 characterized by high antioxidant properties. Gamma- and delta-tocopherols, and their corresponding tocotrienols, are very effective in protecting oils from auto-oxidation. Alfa-tocopherol is a good antioxidant against photo-oxidation. Corn and cottonseed oils are rich in gamma-tocopherol, while sunflower oil contains very little gamma-tocopherol. This may explain why cottonseed oil and corn oil have superior frying stability than the sunflower oil. Tocotrienols contain three unsaturated double bonds as compared with the corresponding tocopherols. This makes tocotrienols more effective as antioxidants against auto-oxidation in the frying process. **(Gupta, 2005).**

The tocopherols and tocotrienols are generally not the major components of vegetable oil, but their presence is vital for stabilizing the unsaturated fatty acids of these oils against oxidative deterioration. Their structures are based on a chroman head with two rings, one phenolic and one heterocyclic, and a phytyl tails isoprenoid side chain at C-2. The phytyl tail is saturated in the case of tocopherols and unsaturated in the case of tocotrienols and other derivatives such as plastochromanol. The four members of each subfamily, i.e., α-,β-, γ-, and δ-, differ from each other in the number and position of methyl groups on the chromanol ring. Naturally, tocopherols occur only as free alcohols, but tocotrienols were mentioned to also occur in esterified forms. The tocopherol molecule has three chiral centers in its phytyl tail, giving the possibility of eight total stereioisomeric forms. Seed oils are mostly dominated by γ- or α-tocopherol, but high levels of Δ-tocopherol especially characterize soybean oil, which is the richest and commercially the most used source of tocopherols. Rice bran and palm oils, on the other hand, represent the important sources of tocotrienols. Rapeseed and linseed oils contain the special, tocotrienol-like, plastochromanol-8. Cereal lipids, namely, wheat germ, maize, and rice bran oils, generally contain very high levels of tocopherols and tocotrienols. Of nut oils, almonds and hazelnut oils are rich in a-tocopherol and pecans and walnuts are rich in γ-tocopherol **(Kamal-Eldin, 2005).**

Schwartz *et al.*, (2008) determined the tocopherol and tocotrienol (i.e. tocol) and plant sterol contents of 14 vegetable and 9 industrial fats and oils available on the Finnish market in 2005 using NP-HPLC with fluorescence detection (tocols) and GC-FID (plant sterols). Best sources of α-tocopherol were wheat germ (192 mg/100 g) and sunflower oil (59 mg/100 g). Oils richest in γ-tocopherol were camelina (72 mg/100 g), linseed (52 mg/100 g) and organic rapeseed oil (51 mg/100 g). Total tocol contents were between 4.2 mg/100 g (coconut fat) and 268 mg/100 g (wheat germ oil). Plant sterol contents ranged from 69 mg/100 g in a frying fat to 4240 mg/100 g in wheat germ oil. Organic rapeseed oil, the second best source of plant sterols, contained 887 mg/100 g. In addition to the target compounds, plastochromanol-8 could be determined in all plant-based samples with contents ranging from 0.13 (walnut oil) to 18 mg/100 g (linseed oil). The lignans sesamin and sesamolin could be identified in sesame oil.

Choe (2013) studied light and temperature effects on tocopherols during the oxidation of sunflower oil. The oxidation was performed at 40, 60, or 80^{o}C for 30, 15, and 6 days, respectively, in the dark or under 1,700 lux light. Oil oxidation was analyzed with peroxide values and conjugated dienoic acid contents, and tocopherols in the oil were separated and quantified by HPLC. The oxidation of sunflower oil increased with temperature increase, and the light decreased the temperature dependence of the oil oxidation. Sunflower oil before oxidation contained tocopherols with a total of 737.96 mg/kg, with α- and γ-tocopherol at 726.41 and 11.56 mg/kg, respectively, and the tocopherol contents decreased during the oil oxidation. Degradation of tocopherols increased with the temperature increase, and its dependence on the temperature was lower under light than in the dark. γ-Tocopherol showed higher stability than α-tocopherol during oxidation of the oil in the dark and under light. Residual amounts of tocopherols showed a relatively good correlation with the degree of oil oxidation. The dependence of α-tocopherol degradation on the oil oxidation was higher than that of γ-tocopherol, and light decreased the dependence on the oil oxidation in both tocopherols; α-tocopherol and γ-tocopherol.

According to **Kamal-Eldin (2005),** several lipid minor components are known to synergize the protective effect of tocopherols and protect the unsaturated fatty acids from being rancid. These include phospholipids and unsaponifiable components such as sterols, carotenoids, and squalene. The phenolic compounds of olive oil have particularly received much attention with respect to their effects on the oxidative stability of the oil. A study based on virgin olive oil, from Cornicabra variety obtained from three successive crop seasons, showed a clear influence of total polyphenols on the oil stability and a much lower contribution of α-tocopherol. Univariate analysis revealed a significant correlation between Rancimat stability and the following compositional parameters: phenols (R = 0.87), ortho-diphenols (R = 0.77), oleic/linoleic ratio (R = 0.71), total tocopherols (R = 0.65), chlorophylls (R = 0.68), and carotenoids (R = 0.59). When a stepwise linear regression analysis was employed, a high multicolinearity was found for total phenols, oleic/linoleic ratio, and tocopherols as having together the maximum correlation with stability.

Chen *et al.* (2011) developed a method based on reversed-phase high-performance liquid chromatography with fluorescence detector for the determination of tocopherols in vegetable oils. Oils were diluted in acetonitrile/tetrahydrofuran (ACN/THF) and injected directly onto HyPurity C_{18} column. Methanol and THF (90:10) mixture was used as a mobile phase. Tocopherols were detected by fluorescence detector. The method had good limit of detection (LOD) (7 ng/g for α-tocopherol and 6 ng/g for β-, γ- and δ-tocopherols) and reproducibility (CV% < 2.8%). The method was applied to the determination of α-, (β+γ)- and δ-tocopherols in extra virgin olive oil (EVOO), sunflower, high oleic sunflower oil (HOSO), hazelnut, soy, corn, peanut and almond oil. A new parameter, the ratio of α-/(β+γ)-tocopherol concentration (named α/β tocopherol ratio), in these vegetable oils was systematically assessed and proposed as a first screening marker of the authenticity of EVOO by studying EVOO/sunflower, EVOO/hazelnut and EVOO/peanut mixtures. The use of δ-tocopherol as adulteration marker was also discussed. This work presented a simple, fast, precise and sensitive method for the determination of tocopherols in vegetable oils. The method was based on a fast sample preparation (1:20 dilution of the oil in ACN/THF mixture, no saponification procedures), direct injection in a low-cost RP HPLC column and high sensitivity fluorimetric detection of tocopherols. Tocopherols are sensitive to light. The procedure proposed for sample preparation can preserve their stability and avoid quantification errors, RP versus NP provides higher column stability, reproducibility of retention times, quicker equilibration and shorter analysis time.

2.3.3- Carotenoid and Chlorophyll Composition

Carotenoids are the yellow-red pigments present in most crude oils, although their presence is often masked by the green color of chlorophyll. They are composed of a long-chain, 40-carbon skeleton, of eight isoprenoid units joined head-to-tail to give a completely conjugated system with alternated double bonds. Biosynthetically, carotenoids are derived from this acyclic long-chain conjugated $C_{40}H_{56}$ structure of lycopene by hydrogenation, dehydrogenation, cyclization and oxidation, or a combination of these processes. The carotenoids can be classified into (1) non-polar unsaturated carotenes with the basic structure of lycopene, and (2) more polar carotenoids, or xanthophylls, with oxygen function at one or both end groups. Red palm oil is the richest source of readily available carotenoids and is, therefore, very useful as a pro-Vitamin A supplement. Crude palm oil contains 500–800 ppm of carotenoids, of which β-carotene and α-carotene account for about 90% (approximately 2:1 w/w), and lycopene, phytoene, and zeacarotenes are *inter alias* the remaining carotenoids. Other vegetable oils contain much lower levels of carotenoids (<100 ppm), but these are removed during the bleaching step in refining. Olive oil contains variable, but low, levels of carotenoids mainly as β-carotene (6–17% of total pigments) and lutein (18–38% of total pigments), but xanthophylls such as neoxanthin, violaxanthin, luteoxanthin, antheraxanthin, mutatoxanthin, and b-cryptoxanthin also occur at low levels **(Kamal-Eldin, 2005).**

Lozano-Sánchez *et al.* (2010) reported that pigments are of two distinct families of compounds, namely chlorophyll and carotenoid pigments. Among these, chlorophylls are the most important. Chlorophylls a and b, and their oxidation products pheophytins a and b, are naturally present in olive oil and contain four pyrrole rings (also called porphyrin) with a magnesium atom in the centre and a long chain alcohol esterified denominated phytol. Pheophytin a is the major components of the chlorophyll. These compounds are responsible for the greenish color in oil. Color is a sensory property with a strong influence on food acceptance. This parameter contributes decisively to initial perceptions about food quality. As with other oils, the color of olive oil depends not only on fruit ripeness, but also on the olive variety, cultivation area, harvest time and processing methods. Moreover, these pigments have an influence on oxidative stability of olive oils. Oxygen and light are two well-known oxidation-producing factors. Chlorophyll and pheophytin have antioxidant and pro-oxidant properties. In the presence of light, both compounds promote lipid oxidation by activated oxygen producing singlet oxygen that decreases the shelf life. However, these pigments are desirable due to their antioxidant nature in the dark. It may be deduced that in the storage conditions of the oils, in darkness, chlorophyll and pheophytin improve the oil stability.

Photo-oxidation plays a significant role in the oxidation of oils rich in chlorophyll and other photosensitizers. However, tocopherols, carotenoids, and polyphenols present in these oils provide considerable protection against light-induced photo-oxidation. These different antioxidants work synergistically and have different protection abilities. The best example for this situation is olive oil. Pheophytin A and β-carotene were found to be sensitive to oxygen, light, and temperature, and α-tocopherol and polyphenols were more stable toward high temperatures. The chlorophyll pigments function as photosensitizers and b-carotene as an efficient quencher both for the excited chlorophylls and singlet oxygen. However, α-tocopherol and polyphenols are needed to protect β-carotene against destruction caused by heat and excessive oxygen. During photo-oxidation, squalene was found to protect α-tocopherol **(Kamal-Eldin, 2005).**

Giuffrida *et al.* (2011) used the reversed-phase high performance liquid chromatography using a C-30 column with photodiode array detection for chlorophyll and carotenoid pigments analyses. In all, 19 compounds were identified and quantified in 60 olive oils samples. The qualitative pigments pattern was similar among the varieties investigated, whereas quantitative differences were found among the different cultivars. In general, pheophytin *a* was the major component (range 0.49–19.42 ppm), followed by β-carotene (range 1.27–9.30 ppm) and lutein (range 0.44–5.12 ppm). Those differences may be due to genetic factors and/or geographical differences. The ratio between the two isochromic pigment fractions, namely the ratio between the chlorophyll and carotenoid fractions showed an average value close to unity. The lutein/β-carotene ratio was less than one in the majority of the cases. These parameters, along with other analytical parameters, could be used as indicators of typicality in olive oils. The presence of a specific pigment profile in olive oils could in fact be used to guarantee the genuineness of the product, since the quality control of food requires a precise knowledge of the pigments composition of the original products.

Kim *et al.* (2012) determined the effects of chlorophyll photosensitization on the oxidative stability of oil-in-water (O/W) emulsions by analyzing headspace oxygen content, lipid hydroperoxides, and headspace volatiles. The roles of transition metals and singlet oxygen were tested by adding ethylene diamine tetra acetic acid (EDTA) and sodium azide, respectively. Emulsions with chlorophylls and visible light irradiation had significantly high lipid hydroperoxides and headspace volatiles and low headspace oxygen content ($p<0.05$) after 32 h while samples without light irradiation did not show any significant changes ($p>0.05$). Sodium azide did not show clear antioxidant capacities in O/W emulsion systems rather showed pro-oxidant properties at some concentration. Addition of EDTA, a metal chelator, accelerated the rates of lipid oxidation in a concentration dependent manner. EDTA may enhance the stability of chlorophylls in O/W emulsions and the resulting higher chlorophyll concentrations may generate more singlet oxygen thus accelerating the rates of lipid oxidation.

2.3.4- Squalene and Other Hydrocarbons

Kamal-Eldin (2005) reported that squalene (2,6,10,15,19,23-hexamethyl-2,6,10,14,18,20 tetracosahexaene), also known as spinacene, is a naturally occurring 30-carbon chain triterpenoid hydrocarbon. Shark liver oil is the richest source of squalene, about 50% by weight. Squalene is present at levels of 0.1–1.2% in olive oils together with low levels of other hydrocarbons. Squalene is found in smaller quantities, <30 mg/100 g, in other vegetable oils. Other hydrocarbons representing a homologous series of saturated C15–C33 chains are also present in vegetable oils. For example, olive oil was reported to contain odd-numbered n-alkanes (C23–C29, 30–180 ppm), a series of C22-C27 n-Δ^9-alkenes, 8-heptadecene 6,10-dimethyl-1-undecene (0.5–2 ppm), as well as about 31 sesquiterpenes (2–37 ppm), the most abundant of which were α-farnesene, α-copaene, eremophyllene, and α-muurolene.

2.4- Phenolic Compounds

Kamal-Eldin (2005) stated that low levels of a wide range of phenolic compounds have been reported to be present in all vegetable oils, which is very important for the oxidative stability of the polyunsaturated fatty acids of these oils. The mostly studied phenolic constituents are perhaps those of olive oil. The level of phenolic compounds in olive oil was reported to vary between 50 and 1000 ppm depending on agronomic and environmental factors, ripeness, processing, and storage conditions of the oil, but they mostly lie in the range of 200–500 ppm. The phenolic compounds of olive oil include the secoiridoid aglycons of oleuropein and ligstroside and the lignans 1-acetoxypinoresinol and pinoresinol. Tyrosol, hydroxytyrosol, and their esters are also major phenols of olive oils and their levels were found to increase during storage of virgin olive oil because of acid-catalyzed hydrolysis of secoiridoid aglycons.

Ocakoglu *et al.* (2009) examined extra virgin olive oils extracted from six olive cultivars examined for their simple phenolics, phenolic acids and flavonoid compounds over two harvest years. Total phenol contents, oxidative stabilities and chromatic ordinates as color parameters were also measured. The most typical phenolic compounds that were identified are hydroxytyrosol, tyrosol, vanillic acid, *p*-coumaric acid, cinnamic acid, luteolin, and apigenin. No significant correlation was observed between oxidative stability and phenolic compounds. Increase in peroxide value over an accelerated oxidation period of 11 days showed weak correlations with total phenol content, vanillin, syringic acid and color parameter a*, as 0.56, 0.55, -0.42, and 0.51, respectively, in terms of correlation coefficient r.

Kamal-Eldin (2005) revealed that the vegetable oils are variably susceptible to free radical auto-oxidation depending on the degree of unsaturation of their fatty acids and their contents of pro-oxidant and antioxidant species. The unsaponifiable fractions of wheat germ, corn, or olive oil are known to retard oxidation in vegetable oils. Trace amounts of metals and metalloproteins occurring in vegetable oils catalyze the oxidation by enhancing initiation and re-initiation by decomposing lipid hydroperoxides. The antioxidant activity of tocopherols is well documented, albeit considerable controversy in connection with the relative antioxidant potency of different tocopherols exists. The commencement of exponential oxidation of vegetable oils is associated with critical minimal concentrations of antioxidants. For example, a significant increase in the rate of oxidation was observed when the concentration of α-tocopherol in olive oil reaches a ‘‘threshold value’’ of 60–70 mg/kg. The main reaction responsible for the antioxidant action of tocopherols is hydrogen atom donation. The antioxidant (AH) tocopherols and tocotrienols scavenge lipid peroxyl radical (LOO*) to form a relatively stable lipid hydroperoxide (LOOH). Then the tocopheroxyl or tocotrienoxyl antioxidant radicals (A*), formed by this reaction, scavenge another peroxyl radical through radical–radical coupling to form a variety of stable secondary oxidation products.

Besides scavenging peroxyl radicals, tocopherols and tocotrienols are also excellent quenchers for singlet oxygen and peroxynitrile. It has been reported that α-tocopherol shows a pro-oxidant activity when present at high concentrations. It was later found that tocopherols do not show a pro-oxidant effect if they are compared with controls devoid of them and other lipid-related antioxidant. However, tocopherols lose their antioxidant efficacy at high concentrations because they participate in a number of reactions that lead to production of alkoxyl radicals that consume tocopherols and initiate new reaction chains because of their low selectivity. Using a kinetic approach, a number of reactions were found to contribute to the loss of tocopherol antioxidant activity that is greater for α-tocopherol than for γ-tocopherol. γ-Tocopherol is generally a better antioxidant for vegetable oils than α-tocopherol, both at low-temperature storage and under thermo-oxidation conditions. It was found that γ-tocopherol degrades at a much slower rate and, thereby, is able to protect the oil longer compared with α-tocopherol **Kamal-Eldin (2005).**

Bakhouche *et al.* (2013) characterized Arbequina extra-virgin olive oils (EVOOs) from different locations in southern Catalonia (Spain) in terms of their phenolic profile, to show the classification of oil samples with respect to geographical area. The phenolic compounds present in 32 olive-oil samples were analyzed by a rapid and effective HPLC–ESI-TOF/MS method, and 18 phenolic compounds belonging to different phenolic types were identified. The results showed no qualitative differences in the phenolic fractions among EVOO from different geographical region. However, quantitative differences were observed in a wide number of phenolic compounds. In all olive-oil samples studied, secoiridoids were the most abundant, followed by lignans, phenolic alcohols, and flavonoids, respectively. Multivariate data were analyzed by canonical discriminant analyses. Seventeen variables were used without a variable reduction step. Phenolic content of extra-virgin olive oils was found to depend highly on geographical area.

Bengana *et al.* (2013) studied the chemical composition of Chemlal EVOO during four harvest dates. Changes occurred in EVOO samples during the maturity process, with a strong effect found in various parameters, especially the MUFA/PUFA ratio, chlorophyll, total phenolic content, and oxidative stability. Indeed, quality parameters studied, such as free acidity, peroxide index, and spectrophotometric absorption (K_{232} and K_{270}) remained within estimated limits of the EU regulation for EVOO. In addition, the individual polar phenols in Chemlal EVOOs were characterized, and 19 phenolic compounds were identified in samples studied using RRLC-ESI-TOF/MS for the first time. No qualitative differences in polar phenolic profile were detected among EVOO samples from different harvest dates. However, significant quantitative differences were found in a wide number of phenolic compounds. Finally, in this study, early harvesting appears more suitable to produce Chemlal EVOO of high chemical quality.

2.5- Relation between Oxidative Stability and Compositions of Edible Oils

Antioxidants are components which prevent auto-oxidation of oils and fats by giving their hydrogen to free radicals formed in the initiation and propagation stages of auto-oxidation. During the past two decades, a lot of researches using natural plants extract in edible oils had been carried out due to the trend to minimize or avoid the use of synthetic food additives. According to the most studies, there were various natural antioxidants which could be extracted from low cost resources, such as most parts of olive plant, green tea, sesame, medicinal plants, etc. One of the most important requirements for a suitable antioxidant in oils and fats was the thermal stability during heat processing. It had been shown that most of natural additives had more antioxidants activity and thermal stability than synthetic ones **(Taghvaei and Jafari, 2013).**

Ceci and Carelli (2010) studied the relation between oxidative stability and composition in 58 virgin olive oils from different cultivars and Argentinean regions over 4 harvest years. The oxidative stability of the oils was assessed using the OSI index (110^oC, 20 L/h air flow). A multiple linear regression model is proposed using OSI values as the dependent variable (multiple $R = 0.933$, $p = 1 \times 10^{-15}$), with positive contributions of the independent variables: fatty acid composition [oleic acid / (linoleic acid + linolenic acid), 55.3%, $p = 1 \times 10^{-15}$], total polyphenols (24.1%, $p = 1.8 \times 10^{-9}$), carotenes (4.8%, $p = 6.1 \times 10^{-5}$), β-tocopherol (1.9%, 6.0×10^{-3}) and other compounds (13.9%). Highly significant correlation was observed between oxidative stability indexes estimated by the compositional model and those experimentally determined by Rancimat method ($b = 0.981$, $R = 0.924$). Chlorophylls and Δ-5-avenasterol contributions to the model were non-significant when variables related with fatty acids and polyphenols were included. The results suggested that the fatty acid composition and the polyphenol content were the main factors that affect the oxidative stability of olive oils. The proposed model allowed the estimation of the oxidative stability in olive oils independently of the cultivar. The model was obtained also taking into account samples that lied out of the international legal limits in some compositional values due to natural variations.

Singh (2013) found that the presence of additional sitosterol limited TG polymer formation in triolein, refined canola, high oleic sunflower and flaxseed oils heated at frying temperature. The anti-polymerization effect of sitosterol had been attributed to the conversion of sterol to steradiene by the 1,2–elimination of water. This fact was further corroborated by the significant increase in TG ester hydrolysis during the 48–72 h period, corresponding with the appearance and growth of the TG polymer peak. The unrefined oils had higher stability at frying temperature compared to refined oils which tended to lose some of the natural components including sterols at various stages of refining, particularly during neutralization and deodorization.

The unsaponifiable fraction in the soap stock generated from the neutralization process had been found to contain on an average 70% sterols while the concentration of sterols in the deodorizer distillate had been reported to range from 2% to 20%. Overall, sterol losses during the complete refining of vegetable oils, had been reported to range between 10% and 70%. Hence, given the effectiveness of sterols, naturally found in vegetable oils, in limiting TG polymer formation at frying temperature, ensuring presence of an optimum level of sterols in refined oils was of critical importance. Also, triolein and vegetable oil samples with 5% additional sterol were found to had the lowest level of TG polymer formation, in comparison to control and samples with 1% and 2% additional sterol, after heating at 180^{o}C for 72 h. Hence, vegetable oils enriched with sterols, at levels higher than those naturally present, might be considered as a means of further enhancing oxidative stability at frying temperatures **(Singh, 2013).**

Baccouri *et al.* (2008) studied the chemical composition and oxidative stability of Tunisian monovarietal virgin olive oils with regard to fruit ripening. The analytical parameters (free acidity, peroxide index and spectrophotometric absorption K_{232} and K_{270}) remained widely within estimated limits of the regulation throughout the maturity process. Nevertheless, this research evidenced that the oleic/linoleic acid ratio showed a decreasing trend as the olives ripened. With regard to phenolic compounds, Chétoui VOO had highest level on oleuropein aglycon and consequently the highest stability value. Finally, in Chétoui EVOO, the anticipate harvest-date, gave the best results in terms of all the parameters considered. Instead, for the cv. Chemlali, the data clearly evidenced higher values of oxidative stability, phenolic and tocopherolic contents between the second and the third harvest-date. All that data could be used to define the ideal ripening degree to confer a good balance between chemical profiles, together with the best oxidative shelf-life of the EVOO.

Quiles *et al.* (2002) studied the effect of lipid profile, vitamin E and total phenolic content in relation to the antioxidant capacity (measured by ESR) of three edible oils (virgin olive, sunflower and olive oils), using short-time deep fat frying as a model. Physico-chemical changes in the oils were also studied. Samples were analyzed before and after 15, 30, 45 and 60 min fryings. Determination of free radicals, by electron spin resonance spectroscopy, revealed the highest antioxidant capacity in virgin olive oil and sunflower oil. This parameter was mainly influenced by vitamin E content, followed by lipid profile and phenolic content. The frying procedure decreased the antioxidant capacity in all tested oils. Sunflower oil underwent more chemical changes by frying than did olive and virgin olive oils. Antioxidant capacity of the edible oils was correlated with polar components and ultraviolet indices but not with peroxide index or acidity value.

Chiou *et al.* (2009) revealed that the supplementation of frying oils with the olive leaf extract was beneficial for the oils, by enhancing their oxidative stability, as a result of higher retention of micro constituents. Supplementation also resulted in elevated amounts of tocopherols, phytosterols and squalene in French fries as compared with those fried in the non supplemented oils.

Yang *et al.* (2013) determined the contents of the minor components and induction period (IP) of cold-pressed oils from 203 rapeseed varieties from the Yangtze River Valley in China. The average contents of total phenolics, phytosterols, tocopherols, β-carotene, lutein and chlorophyll of the samples were 36 mg/100 g, 826 mg/100 g, 378 mg/kg, 2.4 mg/kg, 104 mg/kg and 8.2 mg/kg, respectively. There were significant differences among the varieties in the content of these minor components. Correlation analysis revealed that there was a positive correlation between the IP and the content of total polyphenols, lutein and total tocopherols. Additionally, the IP negatively correlated with total phytosterols and chlorophyll content. Principal component analysis differentiated the rapeseed oils based on their IP and total phenolic, tocopherol, β-carotene, lutein and chlorophyll content.

The antioxidant effects of flavonoids and β-carotene during the thermal auto-oxidation of food relevant oil-in-water emulsions were spectrophotometrically assessed by measuring the formation of primary oxidation products (conjugated dienes and lipid hydroperoxides). An oxidatively “sensitive” model emulsion was selected as substrate of this study in terms of processing and compositional factors. At a concentration of 1.5 mmol kgr^{-1}, only quercetin among the tested compounds significantly reduced the oxidative deterioration of cottonseed oil-in-water emulsions. Structural characteristics (positioning of hydroxyl group) or partitioning behavior between the emulsion phases might modulate the flavonoid activity. The high oxygen pressure conditions of the experimental system may explain the lack of any antioxidant activity for β-carotene. The antioxidant potential of quercetin increased with its concentration until a specific level. On the contrary, the antioxidant concentration within the same tested range (0.75–3 mmol kgr^{-1}) did not impact the activity of catechin and β-carotene. Mixtures of β-carotene with flavonoids did not exert a tendency for increasing the activity of each individual compound **(Kiokias and Varzakas, 2014).**

Roman *et al.* (2013) followed the oxidation kinetics of three vegetable oils (sunflower, high oleic sunflower and rapeseed) during heat treatment (100 °C, 120 °C and 150 °C for 6 h), in the absence or presence of α-tocopherol (around 15×10^{-4} mol L^{-1}) or a mixture of α-tocopherol and chlorogenic acid (around 15×10^{-4} mol L^{-1} of each). The kinetics were followed by measuring several chemical markers: fatty acids, conjugated dienes, hydroperoxides and aldehydes. The results showed that the unsaturation degree of the oils tested was the predominant parameter influencing the progress of oxidation kinetics. In this sense, sunflower oil was systematically more oxidized than rapeseed oil, which itself was more oxidized than high oleic sunflower oil. Oxygenation conditions and oil temperature were also determinant inducers of oxidation. The addition of α-tocopherol significantly delayed the formation of oxidation products while chlorogenic acid did not enhance the oxidative stability of oils but protected α-tocopherol during heating.

2.6- Characterization of Edible Oils by FTIR Spectroscopy

Mossoba *et al.,* (2013) reported that the declarations of the total content of *trans* fatty acids (FA) and saturated FA (SFA) are mandatory on food labels in the US and Canada. Gas chromatography (GC) has been the method of choice for the determination of FA composition. However, GC is time consuming and requires conversion of fats and oils to their FA methyl esters. A recently published Fourier transform near-infrared (FT-NIR) spectroscopic procedure was applied to the rapid (<5 min) determination of total SFA, monounsaturated FA (MUFA), polyunsaturated FA (PUFA), and trans FA contents of 30 commercially available edible fats and oils. Good agreement was obtained between the GC and FT-NIR methods for the determination of total SFA, MUFA, and PUFA contents. Differences between the two methods were apparent for the determination of *trans* fat at *trans* fat levels <2 % of total fat. The analytical determinations of total SFA, MUFA, and PUFA contents form any of the oils examined differed from the respective values declared on the product labels. Their findings demonstrated that the FT-NIR procedure serves as a suitable alternative method for the rapid determination of total SFA, MUFA, PUFA and *trans* FA contents of neat vegetable oils.

Tyburczy *et al.,* (2013) revealed that ATR–FTIR spectroscopy methods have been developed for rapid determination of total *trans* fat in edible fats and oils. These methods have an analysis time of <5 min/sample after generation of the calibration standard linear regression function. Calibration standards are prepared by adding known amounts of trielaidin (*trans*-9 18:1) to a *trans*-free oil at levels covering the expected range of *trans* fat content of the test oils. The calibration standards are analyzed for total *trans* fat content by measuring the height of the negative second derivative or the area of the absorbance band at 966 cm^{-1}, a feature of the mid-IR spectrum (4,000–400 cm^{-1}) that is quantitatively characteristic of isolated *trans* double bonds. The *trans* fat content of test oils is then calculated on the basis of the observed trans band intensity, by use of the calibration standard linear regression function.

2.7- Characterization of Edible Oils by NMR Spectroscopy

Sacco *et al.* (2000) performed analytical measurements and proton nuclear magnetic resonance (^{1}H-NMR) spectra of phenolic extracts on a set of Italian extra virgin olive oils from different cultivars and geographical locations of Apulia region. ^{1}H-NMR data of phenolic extracts permit a classification according to the geographical origin of the samples. To this purpose, ^{1}H-NMR spectra of the phenolic extracts were normalized to the ^{1}H signal of the solvent peak (DMSO-d6). Variable selection was applied to select the resonances having the highest power to distinguish between different oils. The selected variables were normalized intensities of peaks at 9.69, 9.64, 9.52, 9.48, 9.22 ppm (aldehydic protons); 7.57 ppm (vinylic protons); 6.98, 6.77, 6.75, 6.68, 6.64, 6.60, 6.42 ppm (aromatic protons), and 4.70 ppm. A relation between oil varieties and selected ^{1}H-NMR normalized peak intensities also was investigated. A classification of the samples according to their variety with prediction ability of 100% was obtained.

Alonso-Salces *et al.* (2010) presented a new approach to the geographical characterization of virgin olive oils (VOOs) based on the ^{1}H-NMR fingerprint of the unsaponifiable matter. The ^{1}H-NMR spectra of the unsaponifiable fraction of virgin olive oils from Spain, Italy, Greece, Tunisia, Turkey, and Syria were analyzed by several pattern recognition techniques. Moreover, ^{1}H-NMR spectra of the bulk oil, and its corresponding unsaponifiable fraction, as well as the sub-fractions of the unsaponifiable fraction (alcohol, sterol, hydrocarbon, and tocopherol fractions) were studied in the search for the markers that multivariate techniques revealed to be related to the geographical origin of olive oils. Additionally, a preliminary study regarding ^{1}H-NMR data of the bulk oil and the corresponding unsaponifiable fraction of VOOs suggested that these spectral data contained complementary information for the geographical characterization of VOOs.

Olive oil is mainly made up of triglycerides, differing in their substitution patterns in terms of length, degree, and kind of unsaturation of the acyl groups, and by minor components such as mono- and di-glycerides, sterols, tocopherols, aliphatic alcohols, hydrocarbons, fatty acids, pigments, and phenolic compounds. The chemical shifts of the ^{1}H signals of the triglycerides are well known. Minor oil components are only observed by ^{1}H-NMR when their signals do not overlap with those of the main components and their concentrations are high enough to be detected. Some signals of minor compounds were found in ^{1}H-NMR of VOO since they were not overlapping with those of the triglyceryl protons: cycloartenol at 0.318 and 0.543 ppm, β-sitosterol at 0.669 ppm, stigmasterol at 0.687 ppm, squalene at 1.662 ppm, *sn*-1,2-diglyceryl group protons at 3.71 and 5.10 ppm, and three unknown terpenes at 4.571, 4.648, and 4.699 ppm. The signals in the spectra of the unsaponifiable fraction were slightly shifted with respect to the signals in the spectra of the bulk oil (0.013–0.015 ppm to higher chemical shifts). Some signals were due to a particular sub-fraction and/or could also be assigned to individual compounds: cycloartenol at 0.333, 0.558, and 0.974 ppm; β-sitosterol at 0.826, 0.834, 0.848, 0.921, and 0.934 ppm; stigmasterol at 0.702 ppm; and squalene at 1.431 ppm. The NMR spectra of the bulk oil and the unsaponifiable fraction and its sub-fractions provided information about the most influent NMR signals or regions for their classification of VOOs according to the geographical origin **(Alonso-Salces *et al.*, 2010).**

2.8- Methods for Measuring Lipid Oxidation

Numerous analytical methods are routinely used for measuring lipid oxidation in foods. However, there is no uniform and standard method for detecting all oxidative changes in all food systems. Therefore, it is necessary to select a proper and adequate method for a particular application. The available methods to monitor lipid oxidation in foods can be classified into five groups based on what they measure: the absorption of oxygen, the loss of initial substrates, the formation of free radicals, and the formation of primary and secondary oxidation products. A number of physical and chemical tests, including instrumental analyses, have been employed in laboratories and the industry for measurement of various lipid oxidation parameters. These include the weight-gain and headspace oxygen uptake method for oxygen absorption; chromatographic analysis for changes in reactants; iodometric titration, ferric ion complexes, and Fourier transform infrared (FTIR) method for peroxide value; spectrometry for conjugated dienes and trienes, 2-thiobarbituric acid (TBA) value, *p*-anisidine value (*p*-AnV), and carbonyl value; Rancimat and Oxidative Stability Instrument (OSI) method for oil stability index; and electron spin resonance (ESR) spectrometric assay for free-radical type and concentration. Other techniques based on different principles, such as differential scanning calorimetry (DSC) and nuclear magnetic resonance (NMR), have also been used for measuring lipid oxidation. In addition, sensory tests provide subjective or objective evaluation of oxidative deterioration, depending on certain details **(Shahidi and Zhong, 2005).**

2.8.1- Sensory Evaluation

For the food industry, the detection of oxidative off-flavors by taste or smell is the main method of deciding when a lipid-containing food is no longer fit for consumption. Terminologies and methodologies have been developed for sensory evaluation of specific food products such as meats, peanuts, and vegetable oils. In the edible oil industry, the AOCS (American Oil Chemists' Society) Flavor Quality Scale (revised) with separate grading and flavor intensity has been employed for describing lipid oxidation. The descriptive analysis, including the detection and the description of both the qualitative and quantitative sensory aspects of a product, is performed by a trained panel, as the sensitivity to the off-flavors varies among different individuals. The sensory induction period of the product can be determined. Sensory evaluation of lipid oxidation has been conducted by many researchers. However, as a subjective method, the reproducibility of sensory analysis is generally considered worse than that of chemical or instrumental methods. More recently, use of an electronic nose to monitor the formation of volatile compounds associated with off-flavors from lipid oxidation has been proposed to supplement information from human sensory panels **(Shahidi and Zhong, 2005).**

2.8.2- Measurement of Primary Products of Oxidation

2.8.2.1- Peroxide Value (PV)

Lipid oxidation involves the continuous formation of hydroperoxides as primary oxidation products that may break down to a variety of nonvolatile and volatile secondary products. The formation rate of hydroperoxides outweighs their rate of decomposition during the initial stage of oxidation, and this becomes reversed at later stages. Therefore, the peroxide value (PV) is an indicator of the initial stages of oxidative change. However, one can assess whether a lipid is in the growth or decay portion of the hydroperoxide concentration by monitoring the amount of hydroperoxides as a function of time. Analytical methods for measuring hydroperoxides in fats and oils can be classified as those determining the total amount of hydroperoxides and those based on chromatographic techniques giving detailed information on the structure and the amount of specific hydroperoxides present in a certain oil samples. The PV represents the total hydroperoxide content and is one of the most common quality indicators of fats and oils during production and storage. A number of methods have been developed for determination of PV, among which the iodometric titration, ferric ion complex measurement spectrophotometry, and infrared spectroscopy are most frequently used **(Shahidi and Zhong, 2005).**

2.8.2.2- Iodometric Titration Method

Iodometric titration assay, which is based on the oxidation of the iodide ion (I^-) by hydroperoxides (ROOH), is the basis of current standard methods for determination of PV. In this method, a saturated solution of potassium iodide is added to oil samples to react with hydroperoxides. The liberated iodine (I_2) is then titrated with a standardized solution of sodium thiosulfate and starch as an endpoint indicator. The PV is obtained by calculation and reported as milliequivalents of oxygen per kilogram of sample (meq/kg). Although iodometric titration is the most common method for measurement of PV, it suffers from several disadvantages. The procedure is time-consuming and labor-intensive **(Shahidi and Zhong, 2005).**

2.8.2.3- Ferric Ion Complexes

Other chemical methods based on the oxidation of ferrous ion (Fe^{2+}) to ferric ion (Fe^{3+}) in an acidic medium and the formation of iron complexes have also been widely accepted. These methods spectrophotometrically measure the ability of lipid hydroperoxides to oxidize ferrous ions to ferric ions, which are complexed by either thiocyanate or xylenol orange. Ferric thiocyanate is a red-violet complex that shows strong absorption at 500–510 nm. The method of determining PV by colorimetric detection of ferric thiocyanate is simple, reproducible, and more sensitive than the standard iodometric assay, and has been used to measure lipid oxidation in milk products, fats, oils, and liposomes. The ferrous oxidation of xylenol orange (FOX) assay uses dye xylenol orange to form a blue-purple complex with a maximum absorption at 550–600 nm. This method is rapid, inexpensive, and not sensitive to ambient oxygen or light. It can consistently quantify lower hydroperoxide levels; and good agreement exists between the FOX assay and the iodometric method. The FOX method has been successfully adapted to a variety of applications. However, because many factors, such as the amount of sample, solvent used, and source of xylenol orange, may affect the absorption coefficient, knowledge of the nature of hydroperoxides present in the sample, and careful control of the conditions used are required for accurate measurements **(Shahidi and Zhong, 2005).**

2.8.3- Measurement of Secondary Products of Oxidation

The primary oxidation products (hydroperoxides) are unstable and susceptible to decomposition. A complex mixture of volatile, nonvolatile, and polymeric secondary oxidation products is formed through decomposition reactions, providing various indices of lipid oxidation. Secondary oxidation products include aldehydes, ketones, alcohols, hydrocarbons, volatile organic acids, and epoxy compounds, among others. Methods for assessing lipid oxidation based on their formation are discussed in this section **(Shahidi and Zhong, 2005).**

2.8.3.1- Thiobarbituric Acid (TBA) Test

The thiobarbituric acid (TBA) test was proposed over 40 years ago and is now one of the most extensively used methods to detect oxidative deterioration of fat-containing foods. During lipid oxidation, malonaldehyde (MA), a minor component of fatty acids with 3 or more double bonds, is formed as a result of the degradation of polyunsaturated fatty acids. It is usually used as an indicator of the lipid oxidation process, both for the early appearance as oxidation occurs and for the sensitivity of the analytical method. In this assay, the MA is reacted with thiobarbituric acid (TBA) to form a pink MA-TBA complex that is measured spectrophotometrically at its absorption maximum at 530–535 nm. The extent of oxidation is reported as the TBA value and is expressed as milligrams of MA equivalents per kilogram sample or as micromoles of MA equivalents per gram of sample. It must, however, be noted that alkenals and alkadienals also react with the TBA reagent and produce a pink color. Thus, the term thiobarbituric acid reactive substances (TBARS) is now used instead of MA. The TBA test can be performed by various procedures, among which four major types have frequently been employed. These include test on the whole sample, test on an aqueous or acid extract of sample, test on a steam distillate, and test on extracted lipid from a sample. The test on a steam distillate (distillation method) is the most commonly used method for determining TBA value **(Shahidi and Zhong, 2005).**

2.8.3.2- *p* -Anisidine Value (*p* -AnV)

The *p*-anisidine value (*p*-AnV) method measures the content of aldehydes (principally 2-alkenals and 2,4-alkadienals) generated during the decomposition of hydroperoxides. It is based on the color reaction of *p*-methoxyaniline (anisidine) and the aldehydic compounds. The reaction of *p*-anisidine reagent with aldehydes under acidic conditions affords yellowish products that absorb at 350 nm. The color is quantified and converted to *p*-AnV. The *p*-AnV is defined as the absorbance of a solution resulting from the reaction of 1 g of fat in isooctane solution (100 ml) with *p*-anisidine (0.25% in glacial acetic acid). This test is more sensitive to unsaturated aldehydes than to saturated aldehydes because the colored products from unsaturated aldehydes absorb more strongly at this wavelength. However, it correlates well with the amount of total volatile substances. The *p*-AnV is a reliable indicator of oxidative rancidity in fats and oils and fatty foods. A highly significant correlation between *p*-AnV and flavor scores and PV has been found. Nevertheless, some authors have indicated that *p*-AnV is comparable only within the same oil type because initial AnV varies among oil sources. For instance, oils with high levels of polyunsaturated fatty acids might have higher AnV even when fresh **(Shahidi and Zhong, 2005).**

2.8.3.3- Totox Value

The Totox value is a measure of the total oxidation, including primary and secondary oxidation products. It is a combination of PV and *p*-AnV: Totox value = 2PV + *p*-AnV. During lipid oxidation, it is often observed that PV first rises and then falls as hydroperoxides decompose. PV and *p*-AnV reflect the oxidation level at early and later stages of oxidation reaction, respectively. Totox value measures both hydroperoxides and their breakdown products, and provides a better estimation of the progressive oxidative deterioration of fats and oils. However, Totox value has no scientific basis because it is a combination of two indicators with different dimensions. Recently, Wanasundara and Shahidi used TBA values and defined $\text{Totox}_{\text{TBA}}$ as 2PV + TBA using the TBA test in place of the *p*-AnV assay **(Shahidi and Zhong, 2005).**

2.8.3.4- Carbonyls

The carbonyl compounds, including aldehydes and ketones, are the secondary oxidation products generated from degradation of hydroperoxides, and are suggested to be the major contributors to off-flavors associated with the rancidity of many food products. The analysis of total carbonyl compounds, which is based on the absorbance of the carbonyl derivatives, provides another approach to measure the extent of lipid oxidation in fats and oils. In this method, the total carbonyl content is measured by a colorimetric 2,4-dinitrophenylhydrazone procedure. The carbonyl compounds formed during lipid oxidation are reacted with 2,4-dinitrophenylhydrazine (DNPH) followed by the reaction of the resulting hydrazones with alkali. The final colored products are then analyzed spectrophotometrically at a given wavelength. Many variations of this method using an alternative solvent, reagent, wavelength, or workup have been reported **(Shahidi and Zhong, 2005).**

The same author indicated that the determination of total content of carbonyls has been used in different oxidative stability studies. However, it has been criticized because the determination conditions cause degradation of hydroperoxides into carbonyl derivatives, giving erroneous results. Carbonyls produced from protein oxidation may also give rise to higher values than those expected from lipid oxidation alone. The addition of triphenylphosphine (TPP) prior to carbonyl determination has been proposed to avoid the interference from hydroperoxides. Hydroperoxides are reduced by TPP, and neither TPP nor TPPO, the oxidation products of TPP, interfere with the measurement of carbonyl content. In quality assessment of used frying fats, where short-chain carbonyls are already removed by distillation at the high temperature of the deep-frying, selectivity can be improved by determination of higher carbonyl compounds instead of the total carbonyls. HPLC is used to separate the DNPH derivatives of higher carbonyls from those of short-chain carbonyl compounds. Apart from detection of total carbonyl content, the analysis of individual carbonyl compounds has gained popularity for following lipid oxidation. Hexanal, one of the major secondary products formed during the oxidation of linoleic and other ω6 fatty acids, serves as a reliable indicator of lipid oxidation in foods rich in ω6 fatty acids. A strong linear relationship was reported between hexanal content, sensory scores, and TBA values. Moreover, measurement of hexanal offers the advantage of analyzing a single, well-defined end product for antioxidant efficiency studies.

Hexanal can be quantified by chromatography or as the intensity of the carbonyl band by NIR spectroscopy. Nevertheless, these methods may require volatilization of hexanal, whereas hexanal volatilization may be hindered due to covalent or other types of binding between hexanal and proteins in foods and, thus, may affect accurate hexanal quantifications. More recently, an indirect enzyme-linked immunosorbent assay (ELISA) has been developed for monitoring lipid oxidation through quantification of hexanal-protein adducts, which are recognized by polyclonal or monoclonal antibodies. Other carbonyl compounds, including propanal, pentanal, decadienal, etc., are also used for evaluating lipid oxidation in foods. For instance, propanal is a recommended indicator for lipid oxidation in foods that are high in ω3 fatty acids, such as marine oils. In general, it is essential to use appropriate indicators when assessing the oxidative deterioration of different food systems **(Shahidi and Zhong, 2005).**

2.8.3.5- Hydrocarbons and Fluorescence Assay

Formation of saturated hydrocarbons, especially short-chain (C1-C5) hydrocarbons such as ethane, propane, and pentane, can be measured for monitoring lipid oxidation when aldehydes are either absent or undetectable. Pentane content determined by GC techniques, has been a useful parameter to assess rancidity of fats and oils as well as freeze-dried muscle foods. Significant correlations existed between pentane levels and rancid odor scores. It has been observed that the content of secondary oxidation products, such as malonaldehyde (MA), decreases with increased lipid oxidation, which can be explained by further reaction of MA with proteins. MA reacts with compounds containing primary amino groups (proteins, amino acids, DNA, phospholipids) to form fluorescent products. A fluorescence assay has been successfully used to assess lipid oxidation in muscle foods and biological tissues. In addition to MA, hydroperoxides and other aldehydes also react with amino compounds generating various fluorescent products with different excitation and emission maxima. Significant correlations existed between this method and the TBA value as well as oxygen absorption level, and appears to be a reliable indicator of oxidative deterioration in muscle foods, especially in freeze-dried products **(Shahidi and Zhong, 2005).**

2.8.4- Evaluation of Oxidative Stability of Edible Oils by Oil Stability Index (OSI)

Shahidi and Zhong (2005) revealed that during lipid oxidation, volatile organic acids, mainly formic acid and acetic acid, are produced as secondary volatile oxidation products at high temperatures, simultaneously with hydroperoxides. In addition, other secondary products, including alcohols and carbonyl compounds, can be further oxidized to carboxylic acids. The oil stability index (OSI) method measures the formation of volatile acids by monitoring the change in electrical conductivity when effluent from oxidizing oils is passed through water. The OSI value is defined as the point of maximal change of the rate of oxidation, attributed to the increase of conductivity by the formation of volatile organic acids during lipid oxidation. However, this method requires a somewhat higher level of oxidation (PV>100) to obtain measurable results than other methods in which hydroperoxides are the most important products formed and detected. Therefore, to determine oil stability in the laboratory, especially for some oils that are stable under normal conditions, the oxidation process is accelerated by exposing oil samples to elevated temperatures in the presence of an excess amount of air or oxygen. The OSI method differs from ambient storage conditions by using a flow of air and high temperatures to accelerate oxidation. The OSI is an automated development of the active-oxygen method (AOM), because both employ the principle of accelerated oxidation. Nevertheless, the OSI test measures the changes in conductivity caused by ionic volatile acids, whereas PV is determined in the AOM.

Two pieces of commercially available equipment, the Rancimat (Metrohm Ltd.) and the Oxidative Stability Instrument (Omnion Inc.) are employed for determining the OSI value. Rancimat is a rapid automated method, which agrees well with the AOM. In the Rancimat assay, a flow of air is bubbled through heated oil, usually at 100^{o}C or above. For marine oils, temperatures as low as 80^{o}C are often used. Volatile compounds formed during accelerated oxidation are collected in distilled water, increasing the water conductivity. The change of conductivity is plotted automatically and the induction period of the oil or the time taken to reach a fixed level of conductivity is recorded. The Rancimat assay enables continuous monitoring of the oxidation process. Analysis by the Rancimat method is four to five times more rapid than that by the AOM. Excellent correlation between Rancimat and conjugated dienes has been found. However, the main shortcoming of this method is that only eight samples can be included in each batch **(Shahidi and Zhong, 2005).**

Another apparatus, the Oxidative Stability Instrument, operates on the same principle as the Rancimat, and has the capacity of simultaneously analyzing up to 24 samples. Various modifications have been proposed for assessing lipid oxidation by the OSI method. These include the use of auxiliary energies, such as microwaves to shorten the analysis time and a combination of the OSI method with chromatography to obtain specific information about volatile products. The volatiles trapped during measurement by the Rancimat assay can be analyzed by headspace-GC (HS-GC) with FID and GC-MS for quantification of individual volatiles, thus improving the specificity of the assessment. Although the OSI method is useful for quality control of oils, it is not recommended for measurement of antioxidant activity for certain reasons. The high temperatures used do not allow reliable predictions of antioxidant effectiveness at lower temperatures. Volatile antioxidants may be swept out of the oil by the air flow under test conditions, and also the oils are severely deteriorated when endpoint is reached **(Shahidi and Zhong, 2005).**

A comparative study was carried out by **Farhoosh and Yazdi (2014)** in order to evaluate the kinetics of the formation of a number of primary and secondary oxidation products during oxidation of olive oil in the Rancimat test at 100–130°C. They found good correlations between the Rancimat index (OSI) and stability indices (IP) measured in the Rancimat test with no significant differences in kinetic parameters calculated from them. Mean values of the temperature coefficient, Q10 number, activation energy (E_a), frequency factor (A), and free energy of activation (ΔG^{++}) for olive oil oxidation were calculated to be -3.44×10^{-2} $^{o}C^{-1}$, 2.21, 98.91 kJ/mol, 12.17×10^{12} h^{-1}, and 128.25 kJ/mol, respectively. Each unit change in E_a was accompanied by an average 1.43×10^{12} change in A, indicating a higher contribution for factor A than for E_a to the olive oil stability. The E_a and A correlated well with the values of enthalpy and entropy, respectively. The values of OSI or IP could be described well by the ΔG^{++} values. Kinetic data indicated that olive oil stability is more affected by the indigenous antioxidants (tocopherols and/or phenolic compounds) than by the fatty acid composition.

Wang *et al.* (2010) investigated the oxidative stability of diacylglycerol (DAG)-enriched soybean oil and palm olein produced by partial hydrolysis using phospholipase A1 (Lecitase Ultra) and molecular distillation at 110°C by the Rancimat method with and without addition of synthetic antioxidants. Compared with triacylglycerol oils, the DAG-enriched oils displayed lower oxidative stability due to a higher content of unsaturated fatty acids and a lower level of tocopherols. With the addition (50–200 mg/kg) of tert-butylhydroquinone (TBHQ) or ascorbyl palmitate (AP), the oxidative stability indicated by induction period (IP) of these DAG-enriched oils under the Rancimat conditions was improved. The IP of the diacylglycerol-enriched soybean oil increased from 4.21 ± 0.09 to 12.64 ± 0.42 h when 200 mg/kg of TBHQ was added, whereas the IP of the diacylglycerol-enriched palm olein increased from 5.35 ± 0.21 to 16.24 ± 0.55 h when the same level of AP was added. Addition of TBHQ, alone and in combination with AP resulted in a significant ($p \leq 0.05$) increase in oxidative stability of diacylglycerol-enriched soybean oil. AP had a positive synergistic effect when used with TBHQ.

2.8.5- Evaluation of Oxidative Stability of Edible Oils by UV Spectroscopy (Conjugated Dienes, Trienes and DPPH Assay)

It was discovered in 1933 that the formation of conjugated dienes in fats or oils gives rise to an absorption peak at 230–235 nm in the ultraviolet (UV) region. In the 1960s, monitoring diene conjugation emerged as a useful technique for the study of lipid oxidation. During the formation of hydroperoxides from unsaturated fatty acids conjugated dienes are typically produced, due to the rearrangement of the double bonds. The resulting conjugated dienes exhibit an intense absorption at 234 nm; similarly conjugated trienes absorb at 268 nm. An increase in UV absorption theoretically reflects the formation of primary oxidation products in fats and oils. Good correlations between conjugated dienes and peroxide value have been found. Ultraviolet detection of conjugated dienes is simple, fast, and requires no chemical reagents and only small amounts of samples are needed. However, this method has less specificity and sensitivity than PV measurement. Furthermore, the result may be affected by the presence of compounds absorbing in the same region, such as carotenoids. To avoid these interferences, an alternative spectroscopic method measuring conjugable oxidation products (COPs) has been proposed. In this method, hydroperoxides and some decomposition products are converted to more conjugated chromophores by reduction and subsequent dehydration. The concentrations of the resultant conjugated trienes and tetraenes are determined from their respective absorption at 268 nm and 301 nm and expressed as COP values **(Shahidi and Zhong, 2005).**

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay was used to predict the oxidative stability of edible oils. The principle of DPPH method is to measure the free radicals generated from oxidize oils directly. The absorbance of DPPH from oils before thermal oxidation decreased proportionally to the concentration of free radical scavenging compounds such as butylated hydroxytoluene (BHT). Oxidized oils with high polyunsaturated fatty acid (PUFA) contents showed high changes in DPPH absorbance or free radical formation rates than those with low PUFA. Oxidized oils with high BHT content were more stable and showed slower pattern change of DPPH absorbance from positive to negative slopes than those with low BHT content. Oxidative stability of oils could be predicted considering the initial values of DPPH absorbance, the free radical formation rates, and the oxidation period for pattern changes. The results from DPPH method on the oxidative stability of vegetable oils agreed with conventional methods such as *p*-anisidine value (*p*-AV), conjugated dienoic acids (CDA), or total polar matter (TPM). The result of this study can give important information for the researches screening new antioxidant compounds from natural sources through DPPH method. **(Lee *et al.,* 2007).**

2.8.6- Evaluation of Oxidative Satiability of Edible Oils by Electron Spin Resonance (ESR) Spectroscopy (Measurement of Free Radicals)

As reported by **Shahidi and Zhong (2005),** electron spin resonance (ESR), which also referred to as electron paramagnetic resonance (EPR) spectroscopy, relies on the paramagnetic properties of the unpaired electrons in radicals and has been developed for assessing the formation of free radicals originating in the early stages of oxidation and the onset of primary oxidation. The assay measures the absorption of microwave energy when a sample is placed in a varied magnetic field. Quantification of radical concentrations is complicated by comparison with stable paramagnetic compounds, such as transition metals and nitroxyl radicals. However, the short lifetimes and low steady-state concentration of the highly reactive lipid-derived radicals make it difficult to detect these radicals at concentrations lower than the minimum detectable concentration of 10^{-9} M. To overcome this problem, various approaches have been used, including pulse radiolysis and UV photolysis, continuous flow systems and spin trapping, among which spin trapping has been the most widely employed procedure. Spin trapping technique allows the accumulation of detectable concentrations of longer-lived radicals by addition to samples of a spin trapping agent, which reacts with free radicals to form more stable spin adducts, but often at the expense of the ability to identify the original radical. Nitroso compounds and nitrones are the most common spin traps, both leading to nitroxyl type spin adducts, such as α-phenyl-tert-butylnitrone (PBN) adducts.

ESR spectroscopy is of great value for the study of the early stages of lipid oxidation and prediction of oxidative stability of fats and oils. It has high sensitivity and allows mild conditions by applying significantly low temperatures and requires little sample preparation. Strong linear correlations were found between ESR and Rancimat and oxygen consumption analyses. ESR has also been used for evaluation of antioxidant activity. Nevertheless, spin traps used in the ESR assay have been reported to exhibit widely differing trapping efficiencies for different radicals and show both pro-oxidant and antioxidant effects. Moreover, spin adducts can act as antioxidants, giving erroneous results of oxidative stability of samples. However, even with these limitations, the ESR spectroscopy is a suitable method for measuring lipid oxidation in foods and in biological tissues **(Shahidi and Zhong, 2005).**

Papadimitriou *et al.* (2006) evaluated the oxidative stability of extra virgin olive oils (EVOO) from the Greek island of Crete by electron paramagnetic resonance (EPR) spectroscopy and the spin trapping technique. The spin trap *N*-*t*-butyl-α-phenylnitrone (PBN) was added to the olive oil samples and the production of free radicals was monitored during heating at 70°C. Induction time for the accelerated oxidation of virgin olive oils at 70^{o}C was determined. The EPR results were compared with the oxidative stability values provided by the Rancimat method at 110^{o}C and high linear correlations were found (r = 0.922). EPR spin trapping provides a sensitive and rapid method for evaluating the oxidative stability of EVOO.

They also examined the same samples of Greek extra virgin olive oils for their radical scavenging activity (RSA) toward the stable galvinoxyl radical by EPR spectroscopy. The decrease of the intensity of the EPR signal upon incubation time was followed. Both oxidative stability and radical scavenging activity of EVOO samples were correlated to their content in polyphenols and tocopherols.

Several electron spin resonance (ESR) spectroscopy methodologies make it possible to identify, quantify and measure the reactivity of radical species formed during oxidation–reduction reactions. In a study by **Roman *et al.* (2012),** the advantage of the specificity of ESR spectroscopy to detect radical compounds in order to determine the rate constants of hydroperoxide degradation, a key reaction involved in lipid oxidation was taken. The interaction of 5-doxyl stearic acid and lipid-derived radicals was studied by following the intensity of ESR spectra. A kinetic model was developed to simulate data analysis obtained by ESR and values of rate constants for hydroperoxide degradation were determined at 100 and 110^{o}C. This quantitative approach of ESR spectroscopy has produced useful information about new rate estimates for hydroperoxide degradation in edible oils.

2.8.7- Evaluation of Oxidative Stability of Edible Oils by Fourier Transform Infra Red (FTIR) Spectroscopy

Durmaz *et al.* (2010) reported that the FTIR spectroscopy is a recent technique for detecting and quantifying functional groups that arise during the oxidative degradation of lipids. The ATR crystal allows one to work with small oil quantities and eliminate the time consuming KBr disc preparation step. Moreover, due to the open surface to the air, the ATR crystal is very suitable for real-time oxidation experiments. In this study, the formation of different oxidation products was monitored by their characteristic wavenumber regions. Spectral regions were given instead of strict wavenumber values due to the changeable absorbance maxima bands observed for a certain functional group throughout the oxidation period. Unroasted AKO was subjected to oxidation. Several spectral data were collected throughout oxidation experiment but only non-oxidized, medium oxidized and fully oxidized samples' spectra are shown for clarity.

During oxidation, absorbance of some groups increased continuously while others decreased in the course of the oxidation period. There were increases and decreases in some of other wavenumber regions but they evaluated only the regions that are known to be due to certain oil oxidation products. The wavenumber region of 700–725 cm^{-1} belongs to *cis* double bonds in unsaturated fatty acids. During oxidation, due to the loss of *cis* double bonds, the absorbance intensity in this region decreases, as they observed in this study. An increase in absorbance that they observed at the region of 960–975 cm^{-1} was probably the result of the formation of *trans* double bonds that arose in the course of heat induced oxidation. Secondary oxidation products such as aldehydes and ketones are known to have absorption in the 880–890 cm^{-1} and 1,690–1,700 cm^{-1} regions. Thus, the absorbance increase they observed near these regions could be attributed to the accumulation of secondary oil oxidation products. A reduction in the absorbance near the 3,008–3,010 cm^{-1} wavenumber region was possibly due to the disappearance of *cis* double bonds as in the case of the 700–725 cm^{-1} band. The absorbance increase near the 3,400–3,600 cm^{-1} region was reported to be caused by the formation of hydroperoxides and alcohols. Generally it was accepted that 3,444 cm^{-1} is a characteristic band for hydroperoxides **(Durmaz *et al.*, 2010).**

Yahuaca-Juárez *et al.* (2013) evaluated the effect of alkaline cooking on the oxidative stability of oil in corn flour. A central composite design was used to study the combined effect of lime concentration (%) and steep time (h) on peroxide value (PV); specific extinction coefficients at 232 and 270 nm (K_{232} and K_{270}); and FTIR absorbance at 3009 cm^{-1}, 3444 cm^{-1}, and 3530 cm^{-1} in oils from corn flour obtained by alkaline cooking. The results indicated that lime concentration and steep time affected the PV, K_{232}, and K_{270}. A decrease of 2.56 % was observed in the IR absorption bands, corresponding to the polyunsaturated fatty acids. The processed-corn oil samples showed a decrease in the band at 3009 cm^{-1} compared to the control, which can be attributed to the loss of the double bonds of the linoleic acid. Based on this observation, the area under the curve was calculated from corn oil samples. The value obtained for the control corn oil sample was 0.156, whereas the mean of the corn oil samples processed was 0.152, indicating a decrease of 2.56 %. The band at 3009 cm^{-1} is attributed to the C-H tension vibrations at the *cis* double bonds (=CH) of the polyunsaturated fatty acids. Corn oil can be oxidized, resulting in a decrease in the concentration of unsaturated acyl groups, which are either fragmented to yield compounds of lower molecular weight, or react with other acyl groups forming dimers, oligomers or polymers.

The FTIR spectra also showed absorption bands related to the secondary oil oxidation products. Oxidation processes in oils rich in linoleic acids involve the formation of hydroperoxides and (Z,E) and (E,E) conjugated-dienic systems. These compounds evolve to give secondary oxidation products resulting from the fragmentation of triglyceride chains, which yield compounds of low or high molecular weight. During the oxidation process, the hydroperoxide increases (around 3444 cm^{-1}) as the corresponding IR absorption. In the advanced stages of oxidation, a new band appears at 3530 cm^{-1}, reflecting the formation of alcohols (secondary oxidation products). In general, the processed-corn oil samples showed bands of higher intensity when compared to the control sample, related to hydroperoxides (3444 cm^{-1}) and secondary oxidation products (3530 cm^{-1}). This finding coincides with the reduction in band intensity observed at 3009 cm^{-1}, related to linoleic acid **(Yahuaca-Juárez *et al.*, 2013).**

Allendorf *et al.* (2012) carried out a study to evaluate the capabilities of a handheld mid-infrared (MIR) spectrometer combined with multivariate analysis to characterize oils, monitor chemical processes occurring during oxidation, and to determine fatty acid composition. Vegetable oils (corn, peanut, sunflower, safflower, cottonseed, and canola) were stored at 65^oC for 30 days to accelerate oxidation reactions. Aliquots were drawn at 5 day intervals and analyzed by benchtop and portable handheld mid-infrared devices (4,000–700 cm^{-1}) and reference methods (IUPAC 2301, 2302; AOCS Cd 8-58; and Shipe 1979). PLSR and soft independent modeling of class analogy (SIMCA) models were developed for oil classification and estimation of oil stability parameters. Models developed from MIR spectra obtained with a benchtop spectrometer equipped with a 3-bounce ATR device resulted in superior discriminative performances for classifying oils as compared to those obtained from handheld spectra (single-bounce ATR). Models developed from reference tests and handheld spectra showed prediction errors (SECV) of 1 meq/kg for peroxide value, 0.09% for acid value and 2% for determination of unsaturated fatty acids in different oils.

The most prominent absorption regions were the –C=CH *cis*, CH_2, and CH_3 stretching vibrations between 3,050 and 2,800 cm^{-1}, stretching vibrations of double bonds (–C=O of esters) between 1,750 and 1,650 cm^{-1}, bending and rocking vibrations of CH, CH_2, and CH_3 groups between 1,465 and 1,370 cm^{-1}, and the –C–O stretching and CH_2 rocking vibrations in the fingerprint region (1,250–700 cm^{-1}). Hydroperoxides (O–H) absorbs between 3,470 and 3,445 cm^{-1} and ester bond of free fatty acids absorb at 1,711 cm^{-1}. The major differences between the oils were in the fingerprint region between 1,200 and 1,000 cm^{-1}, with a prominent band at ~1,100 associated to –C–O bond ester asymmetric stretching vibrations. In terms of oil composition, these correspond to differences in esters derived from secondary alcohols (1,100 cm^{-1}) present in triglyceride molecules. Spectral regions ~3,012–2,850 cm^{-1} (C–H stretching bands/shoulders of fatty acids), ~1,740 cm^{-1} (C=O stretching of esters), and ~1,114 cm^{-1} (–C–O stretching) were found to be important for prediction. After 30 days of stressing the oils at 65°C, the oils showed significant spectral differences from the fresh oil. Differences in chemical structures responsible for discrimination were different from those of fresh oils attributed to thermal stress. The main discriminating band in the thermally abused oils was 1,740 cm^{-1}, associated with stretching vibrations of the ester (–C=O) linkage. Changes in the chain length due to oxidation of fatty acids at the double bonds was also evident from the increase in discriminating power at 2,920 cm^{-1} ($–C–H_2$ asymmetric stretching) and 2,850 cm^{-1} ($–C–H_2$ symmetric stretching). Handheld-FTIR instruments combined with multivariate-analysis showed promise for determination of oil quality parameters **(Allendorf *et al.*, 2012).**

2.8.8- Evaluation of Oxidative Stability of Edible Oils by Nuclear Magnetic Resonance (NMR) Spectroscopy

The nuclear magnetic resonance (NMR) spectroscopy is a powerful method for elucidating the structure of a molecule. Kinetic studies of reactions at temperatures in the range from −150 to 200°C are another application. In solvent systems an understanding of the basic types of solvents present can be had by scanning the product in the NMR region. Of course, the most important applications of NMR spectroscopy are in organic syntheses to determine the class of compounds to which the molecule belongs and to provide more detailed information about its structure. Integration of areas under the absorption peaks and the peak of the internal standard enables quantitative analysis to be performed. The nuclei of certain isotopes have an intrinsic spinning motion around their axes that generates a magnetic moment along the axis of spin. These nuclei include ^{1}H, ^{13}C, ^{19}F, ^{29}Si, and ^{31}P. The simultaneous application of a strong external magnetic field and the energy from a second and weaker radio-frequency source to the nuclei results in the rotation of the macroscopic nuclear magnetization away from its equilibrium position parallel to the applied magnetic field. Absorption occurs when these nuclei undergo transition from one alignment in the applied field to an apposite one. The energy needed to excite these transitions can be measured by the resonance frequency that causes the transitions between energy levels **(Patnaik, 2004).**

High-resolution ^{1}H NMR spectroscopy, in which hydrogen atoms (proton, ^{1}H) with various locations in the triacylglycerol (TAG) molecules are determined, has been used to evaluate oxidative deterioration of fats and oils. The principle of NMR is that hydrogen atoms in a strong magnetic field absorb energy, in the radiofrequency range, depending on their molecular environment, in which changes occur during the oxidation process. These changes may be monitored by NMR spectroscopy as a reflection of oxidation level of food lipids. The oil sample is dissolved in $CDCl_3$ to avoid inference from solvent, and its NMR spectrum recorded, with tetramethylsilane (TMS) as an internal standard. The spectrum shows several groups of signals, corresponding to the hydrogen atoms in different locations in the TAG molecules. The total number of each of these differently located protons can be calculated, from which ratios of aliphatic to olefinic protons (Rao) and aliphatic to diallylmethylene protons (Rad) may be obtained. Both ratios increase steadily during lipid oxidation and may serve as an index of oxidative deterioration of oil samples **(Shahidi and Zhong, 2005).**

NMR spectroscopy has been used by many researchers, and the changes in Rao and Rad measured by NMR correlated well with Totox values, conjugated diene values, and TBA values. In addition to ^{1}H-NMR, ^{13}C-NMR and ^{31}P-NMR are also powerful tools to predict oxidative stability of oils. ^{13}C-NMR enables direct observation of carbon atoms.

The selectivity and dispersion of ^{13}C-NMR spectra are very high. ^{13}C-NMR assesses lipid oxidation by monitoring the changes of carbon chains in TAG molecules, revealing the specific sites that oxidative degradation occurs. However, because the abundance of the NMR active ^{13}C nucleus isotope is only 1.12% of ^{12}C, the sensitivity of ^{13}C-NMR is usually much lower than that of ^{1}H-NMR. NMR spectroscopy is a rapid, nondestructive, and reliable technique for assessing lipid oxidation. It simultaneously measures both the primary and the secondary oxidative changes in oils, and provides specific information on oxidative regions in the TAG molecules. Thus, NMR spectroscopy is considered a more suitable means for estimating lipid oxidation than chemical determinations **(Shahidi and Zhong, 2005).**

Lipid oxidation includes a complex set of chemical reactions; and no single analytical method is available to give a satisfactory description of lipid oxidation status. **Falch *et al.* (2004)** tested the high-resolution NMR spectroscopy techniques were tested to establish possible correlations with traditional analytical methods and to study lipid oxidation products. Ethyl esters of all-*cis* 4,7,10,13,16,19-docosahexaenoic acid (DHA) (22:6n-3), with and without added α-tocopherol, were oxidized in the dark at 25°C in an air-circulating oven. Correlations were found between primary oxidation products (PV and conjugated dienes) and the appearance of peaks in the 8.0–10.5 ppm chemical shift region of the ^{1}H-NMR spectra. Multivariate data analysis (partial least squares; principal component regression) and the study of specific regions of the spectra obtained made it possible to easily separate samples of esters with and without α-tocopherol based on the oxidation products formed. Based on knowledge about lipid oxidation products formed in marine lipids, selected oxidation products have been studied in the ^{1}H-NMR spectra with the aim of finding detection limits and their chemical shift values.

Guillén and Ruiz (2005) studied the oxidation process of olive, hazelnut, and peanut oils at 70 °C with circulating air by ^{1}H-NMR. The evolution of the process, including the rate of degradation of the acyl groups and of formation and degradation of primary oxidation products, as well as the rate of formation of secondary oxidation products such as aldehydes, was commented on and compared with the oxidation of oils rich in polyunsaturated acyl groups. Differences that were due not only to the rate of the processes but also to the nature and concentration of the generated aldehydes were observed. Oils rich in monounsaturated acyl groups generated smaller amounts of the geno- and cytotoxic oxygenated aldehydes than oils rich in polyunsaturated acyl groups.

Guillén and Ruiz (2006) in another assay, the oxidation processes of virgin olive, corn and linseed oils, repeatedly submitted to the effect of microwave heating until the temperature reached 190°C, had been studied by means of ^{1}H-NMR. These oxidation processes were very different to those produced at 70°C with aeration; differences had been found not only in the rate of degradation and in the oxidation mechanisms but also in the nature and proportions of the aldehydes generated. It had also been shown that these oxidative conditions affect the three oils studied in very different ways; virgin olive oil was the least affected by this process and in addition its degradation produced lower proportions of harmful aldehydes.

Khallouki *et al.* (2008) studied food and cosmetic grades of argan oil by ^{1}H-NMR. Peroxide and hydroperoxide measurements as well as low field ^{1}H-NMR experiments showed that both grades offer a good resistance against auto-oxidation. The NMR data showed a high ratio of fatty acid aliphatic to bisallylic CH_2 groups (much higher in argan oils than other vegetable oils), which may contributed to the argan oxidative stability. On one hand, ^{1}H-NMR experiments showed that in argan oils valuable minor components, such as linolenic fatty acid or β-sitosterol, were either not present at all or present in extremely low and undetectable amounts, whereas they are systematically detected in other vegetable oils, such as olive oils.

Guillén and Goicoechea (2009) stored oil liquid matrix of several corn oil samples at room temperature in closed receptacles for different periods of time to be studied by means of ^{1}H-NMR, in order to further knowledge about this type of edible oil oxidation. As expected, the degradation of linoleic acyl groups predominated. In samples at early oxidation stages the presence of hydroperoxides and of (*Z,E*) conjugated-dienic systems was demonstrated, the concentration of the first group being higher than that of the second. In addition to these compounds, the presence of hydroxy derivatives supporting (*Z,E*) conjugated-dienic systems, as well as of hydroperoxy derivatives supporting (*E,E*) conjugated-dienic systems, in samples at intermediate and advanced oxidation stages, was also shown. Corn oil samples at advanced stages of oxidation also contained aldehydes, among which there were alkanals, (*E*)-2-alkenals, (*E,E*)-2,4-alkadienals, 4-hydroxy-(*E*)-2-alkenals, 4-hydroperoxy-(*E*)-2-alkenals and 4,5-epoxy-(*E*)-2-alkenals. The concentrations of the different kinds of intermediate compounds above mentioned as well as of the different kinds of aldehydes present in the oil liquid matrix were determined. These latter compounds can be either free or joined to truncated structures of triglycerides. In addition, a principal component analysis between storage conditions and oxidation level of the samples was carried out.

Alonso-Salces *et al.* (2011) used ^{1}H-NMR fingerprinting to evaluate the stability of olive oil at room temperature while protected from light. Principal component analysis (PCA) of the ^{1}H-NMR spectral data allows to visualize the evolution of virgin olive oil (VOO) with time. The PCA loadings disclose the chemical compounds responsible for the compositional changes taking place in VOO. None of the ^{1}H-NMR signals present at time zero disappeared or experienced significant decreases or increases within the 3 years and seven months of the study.

However, some small changes in the signals and the appearance of some low intensity ones, indicate that some oxidative and hydrolytic degradation of the VOO started after one year. The presence of ^{1}H-NMR signals of hydroperoxides (primary oxidation products) with relative small intensities indicates that the oxidative degradation was taking place at a very low rate and yield. Moreover, the characteristic resonances of aldehydes (main secondary oxidation products) were not detected in the VOO over the studied time period; therefore the secondary oxidation process had not yet occurred. These results confirm the high oxidative stability of VOO at room temperature. On the other hand, slight changes in the ^{1}H-NMR signal intensity of triglycerides and *sn*-1,3-diglycerides indicates that some hydrolytic degradation of the VOO had started.

Cordella *et al.* (2012) studied the degradation process of edible oils of different nature, submitted to heating at 170^{o}C, 190^{o}C and 210^{o}C with aeration, by means of ^{1}H-NMR. In this study, secondary products such as aldehydes were detected and monitored over time. Two complementary analytical approaches were adopted to characterize the kinetics of the appearance of aldehydes in the heated oils. This first was a classical kinetic approach based on the assumption that the overall degradation reaction to form aldehydes follows a rate law of order 1. This approach allowed them to calculate a thermal stability criterion for classifying the oils according to their heat stability. A second approach was to use the spectral fingerprint corresponding to aldehydes in a multivariate data analysis procedure in order to give the major trend in the studied phenomena, taking into account the multiway nature of recorded data. The application of different 3-way and 4-way Tucker3 models led to a better understanding of the chemical stability of the oils studied and was used to determine the order of stability of these oils. This multiway approach provided additional information that 2-way processing (PCA) did not provide clearly, such as the overall contribution of the heating time factor on the chemical evolution of oils. This work showed that a fully chemometric study of NMR spectra allowed to order the oils according to their thermal stability and to achieve a result in good agreement with existing analytical and kinetic studies in the literature.

Guillén and Uriarte (2012) carried out a study to demonstrate the potency of ^{1}H-NMR in monitoring the changes in the composition of virgin linseed oil heated at frying temperature. Linseed oil was heated at frying temperature in an industrial fryer for 20 h. The evolution of its composition was monitored by ^{1}H-NMR. The composition parameters determined simultaneously were: the molar percentage of the different acyl groups, the iodine value, the concentration of the total aldehydes as well as of the different kinds of aldehydes; in addition, the percentage in weight of polar compounds was also determined at different heating times. When heating this oil the molar percentage of linolenic groups diminished at an important rate while that of linoleic or di-unsaturated groups increased, as did that of saturated plus modified groups, that of oleic or monounsaturated groups remaining unchanged. The main aldehydes formed were (*E*,*E*)-2,4-alkadienals together with n-alkanals and (*E*)-2-alkenals. This oil reached the safety limit of 25% in weight of polar compounds very early with a very small degradation level of acyl groups and a very low content of aldehydes. The changes undergone by this oil were compared with those of sunflower oil and of extra virgin olive oil submitted to the same degradative conditions. It had been proved that the highest rate of increase in the percentage in weight of polar compounds per hour of heating was produced in extra virgin olive oil whereas the smallest was produced in virgin linseed oil. This could result from the difference of polarity between the original and the newly formed compounds being higher in the first oil than in the second one. In fact, virgin linseed oil reached the safety limit of 25% in weight of polar compounds after 3.6 h of heating whereas extra virgin olive oil reached this safety limit after 33.7 h. Finally, it only remains to add that very different compositions in heated oils correspond to a content of 25% in weight of polar compounds.

Skiera *et al.* (2012) reported that within the course of lipid peroxidation, hydroperoxides are formed as primary products. They can be used as analytical markers to assess the deterioration status of oils and fats. Here a new ^{1}H-NMR assay to determine the hydroperoxide amount in edible oils was presented. They were able to show that the analytical performance of the method is similar to that of the commonly used peroxide value (PV) according to Wheeler. A total of 290 edible oil samples were analyzed using both methods. For some oil varieties considerable discrepancies were found between the results obtained. In the case of black seed and olive oil, two substances could be identified that cause positive (black seed oil) and negative (olive oils) deviations from the theoretical PV expected from the NMR values.

2.8.9- Other Methods for Measuring Lipid Oxidation

2.8.9.1- Evaluation of Oxidative Stability of Edible Oils by Chemiluminescent Method

One of the most important quality indices of edible oils is the total hydroperoxide content expressed as peroxide value (PV). Oils with high hydroperoxide content show a degree of oxidation and, hence, lower quality. For these reasons, the development of methods for the evaluation of this quality index of edible oils is required. **Tsiaka *et al.* (2013)** aimed to develop, a rapid, accurate, sensitive, simple and low cost chemiluminescence (CL) method for the determination of the total hydroperoxide content of different kinds of olive oils and other types of edible oils. The CL method proposed is based on the chemiluminescent reaction of alkaline luminal and the hydroperoxides of oil, catalyzed by Fe (III) using 1-propanol as the reaction solvent. Calibration curves of the CL intensity as a function of concentration of di-tert-butyl peroxide, used as an external peroxide standard, and of different types of edible oils were prepared. In all the cases the correlation coefficient (R) of the regression lines was satisfactory (R > 0.996). The precision of the method expressed in terms of repeatability and reproducibility was also satisfactory, as repeatability in terms of mean %RSD was 4.8% and reproducibility in terms of mean %RSD was 8%. The method was applied for the evaluation of total hydroperoxide content of olive oils, corn oils, sunflower oils, sesame oils and soybean oils, within the concentration range of 0.1–9.0%v/v and the obtained results were compared with those of the official method for peroxide value. Finally, the different types of olive oils and seed oils had been classified according to their estimated total hydroperoxide content.

2.8.9.2- Evaluation of Oxidative Stability of Edible Oils by Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry (DSC) monitors the changes of physical or chemical properties of a material as a function of temperature by detecting the heat changes associated with phase transitions such as crystallization and glass transition. DSC compares the rate of heat flow of a sample to that of an inert reference material as both are heated or cooled. DSC thermograms are characterized by endothermic and/or exothermic peaks whose area is proportional to the enthalpy gained or lost by the material undergoing phase transition. The phase transitions take place over a specific temperature range depending on the sample compositions and physical properties **(Vittadini *et al.*, 2003).**

Chiavaro *et al.* (2008) reported that DSC has advantages over the more classical detection methods, as it is rapid and does not require sample preparation or solvent utilization. The application of DSC to the analysis and characterization of oils and fats for the determination of solid fat content, crystallization and melting profiles, enthalpy of transitions and polymorphic forms, is well known and reviewed. DSC application has also been suggested as a valuable tool for characterization of oils from vegetable sources and thermal parameters have been reported to correlate well with chemical parameters obtained with standard methods.

Zhang *et al.* (2014) revealed that flaxseed oil comes from flaxseed or linseed is recognized and accepted as healthful and edible oil with an outstanding content of alpha-linolenic acid (ALA). The effect of heating on the thermal properties of flaxseed oil extracted from flax seeds had been investigated. The flaxseed oils were heated at a certain temperature (75, 105, and 135^{o}C) for 48 h. The melting curve (from -75 to 100^{o}C) of flaxseed oil was determined by DSC at intervals of 4 h. Three DSC parameters of exothermic event and endothermic event, namely, peak temperature (T_{peak}), enthalpy, and temperature range were determined. The initial flaxseed oil exhibited an exothermic peak, two endothermic peaks, and two endothermic shoulders between -68 and -5^{o}C in the melting profile. Heating temperature had a significant influence on the oxidative deterioration of flaxseed oil. The melting curve and parameters of flaxseed oil were almost not changed when flaxseed oil was heated at 75^{o}C. However, the endothermic peaks of melting curve decreased dramatically with the increasing of heating time when heating temperature was above 105^{o}C. There is almost no change of melting heat flow of flaxseed oil when heating time exceeded 32 h at 135^{o}C. The preliminary results suggest that the DSC melting profile can be used as a fast and direct way to assess the deterioration degree of flaxseed oil.

Gardette and Baba (2013) characterized the oil extracted from the bean of *Balanites aegyptiaca* (Toogga oil), and they evaluated its photochemical and thermal stabilization. The chemical composition was determined using gas chromatography (GC), revealing that the oil is very rich in unsaturated fatty acids (72% omega-6 and omega-9). The photochemical stability was assessed by subjecting it to artificially accelerated photo-aging and then examining the changes using infrared spectroscopy. The thermal stability was studied at six different temperatures ranging from 130 to 200^{o}C and monitored in situ by DSC.

The kinetic parameters (E_A and k) describing the thermal degradation of this oil were calculated. It had been shown that the antioxidants present in the oil delay the oxidation process (induction period). The degradation of the Toogga oil was compared with that of oleic and linoleic fatty acids. In addition, the degradation of the Toogga oil extracted with hexane was compared to that of the neat oil.

Pardauil *et al.* (2011) applied DSC and a Rancimat method apparatus to evaluate the oxidative stability of buriti pulp oil *(Mauritia flexuosa Mart)*, rubber seed oil *(Hevea brasiliensis)*, and passion fruit oil *(Passiflora edulis)*. The Rancimat measurements taken for the oxidative induction times were performed under isothermal conditions at 100°C and in an air atmosphere. The DSC technique involved the oxidation of oil samples in an oxygen-flow DSC cell. The DSC cell temperature was set at five different isothermal temperatures: 100, 110, 120, 130 and 140°C. During the oxidation reaction, an increase in heat was observed as a sharp exothermic curve. The value T_0 represents the oxidative induction time, which is determined from the downward extrapolated DSC oxidative curve verses the time axis. These curves indicate a good correlation between the DSC T_0 and oxidative stability index (OSI) values. The DSC method is useful because it consumes less time and fewer samples.

Vegetable oils of different types and qualities are widely used in homemade cooking and food industry. Most of the safety concerns were related to possible oxidation processes produced at the relatively high temperatures used when frying. Thus, the thermal stability to oxidation is an important parameter for edible oils. **López-Beceiro *et al.* (2011)** studied oils from the Arbequina, Picual, Hojiblanca and Cornicabra olive varieties, corn, soybean and sunflower by means of pressure differential scanning calorimetry (PDSC). The general aim of this work was to evaluate the thermo-oxidative stability of these vegetable oils by the ASTM onset oxidation temperature (OOT) method. In addition, the ability of some parameters to identify different oils and some relations between the chemical composition and the OOT results were investigated.

2.9- Methods for Measuring Antioxidant Activity

A variety of natural and synthetic antioxidants are used in fat-containing foods in order to inhibit lipid oxidation with a wide range of efficiencies, depending on their properties, concentrations, and processing conditions. Although numerous methods have been proposed for measurement of antioxidant activity, the essential features of any test are a suitable substrate, an oxidation initiator, and an appropriate measure of endpoint. Therefore, certain aspects should be taken into consideration when selecting a test for measuring antioxidant activity. These include the model food system used for the test, and the means by which oxidation is accelerated and monitored. Normally, most assessments of antioxidant activity are performed in oil, or other model systems, giving sensible prediction for the activity in oil or water-in-oil emulsions, whereas the results may be misleading for oil-in-water emulsions. Furthermore, stripping of oils may be necessary in such evaluations because the endogenous antioxidants in non-stripped oils are found to enhance the oxidative stability of oils, thus giving rise to erroneous results in the efficiency of antioxidants under investigation. In addition to oils and fats, lipid substrates used for testing antioxidant activity could be fatty acids, fatty acid ethyl esters or triacylglycerols, and β-carotene. Most test procedures involve initiators to accelerate oxidation **(Shahidi and Zhong, 2005).**

The same authors added that the combination of increased temperature and oxygen supply, addition of metal catalysts, and exposure of the reactants to light can reduce the oxidative stability by a large amount. Nevertheless, the elevated temperature may bring about changes in the oxidation mechanism, thus causing difficulties in the prediction of antioxidant activity at low temperatures as compared with those at high temperatures. After the substrate is oxidized under standard conditions, the oxidation is monitored by chemical, instrumental, or sensory methods. An appropriate measure of endpoint is essential for assessing antioxidant activity. Analytical strategies for endpoint determination include measurement at a fixed time point, measurement of reaction rate, lag phase measurement, and integrated rate measurement. The resulting antioxidant activity is expressed using a wide range of parameters.

Approaches proposed for testing antioxidant activity include measuring of the current state of oil samples, as discussed above, and radical scavenging assays, which are gaining popularity in the evaluation of antioxidant activity. Radical scavenging methods measure the relative abilities of antioxidants to scavenge synthetic radicals or natural in comparison with the antioxidant potency of a standard antioxidant compound. Trolox (6-hydroxy-2,5,7,8-tetramethylchroma-2-carboxylic acid), ascorbic acid, and quercetin are among the standard antioxidants frequently used. The most commonly used synthetic radicals are DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (3-ethylbenzthiazoline-sulfonic acid) radicals. DPPH test and ABTS assay are simple, rapid, and involve no substrate. However, it has been suggested that these artificial substrate-free methods do not always adequately mimic the processes in food systems, which sometimes makes them less valuable for predicting the effectiveness of the antioxidant in foods. Other measurements of antioxidant activity include FRAP (ferric reducing-antioxidant power), TRAP (total radical-trapping antioxidant parameter), phycoerythrin assay, and test of metal chelating capacity **(Shahidi and Zhong, 2005).**

3- MATERIALS AND METHODS

3.1- MATERIALS

3.1.1- OILS

Fresh olive fruits *(Coratina olive variety of early ripe stage)* were brought during seasons of 2012 from Horticulture Research Institute Farm (Giza). Moringa seeds *(Moringa oleifera variety)* were brought during seasons of 2012 from Desert Research Center Farm in El-Sheikh Zowaid. Apricot kernels were brought during seasons of 2012 from Fruit Drying Factory in Food Technology Research Institute (Giza). Crude sunflower oil (SO) was obtained from Arma Oil Industries (10th of Ramadan City, Sharqia, Egypt).

3.1.2- REAGENTS

All of the reagents (analytical and HPLC grade) and the standard solutions were obtained from Sigma Chemical Co.

3.2- METHODS

3.2.1- Oil Extraction

3.2.1.1- Extra virgin olive oil

The extra virgin olive oil (EVOO) was extracted from the fresh olive fruits by pressing using Oliomio-machine with capacity of 30 Kg olive fruits/hour in Olive Research Department/Horticulture Research Institute (Giza). The Oliomio-machine consists of metal crusher unit, malaxation unit, and two-phases centrifugal decanter which are all working to separate the olive paste into vegetable water and pomace together from an one outlet and olive oil from the other outlet, then filtered through a filter paper (Whatman No. 1).

3.2.1.2- Moringa and apricot kernel oils

Moringa seeds were air-dried at room temperature for 1 week, then cleaned, dehulled and crushed. Whereas, apricot kernels were washed thoroughly with water and then air-dried at room temperature for

1 week. The fruit stones were removed and the inner kernels were crushed. Each of crude moringa oil (MO) and apricot kernel oil (AKO) was then extracted by soaking the crushed seeds in hexane (BP 40-60^{o}C) at a ratio of 1:2 (w/v), three times (approximately 24 hours each time). The solvent extract was then filtered, dried over anhydrous sodium sulfate and desolventized under vacuum at 40^{o}C using rotary evaporator.

All the extracted crude oils (EVOO, MO, AKO) and the obtained SO were kept in brown bottles at -5^{o}C till analysis.

3.2.2- Analytical Determinations

3.2.2.1- Refractive Index (RI)

The refractive index of oils was determined according to the method described in **AOAC (2005)** using Carl Zeiss refractometer, and the results were standardized at 25^{o}C.

3.2.2.2- Color Index

The color of each of the oils was measured using a Lovibond tintometer according to the method (Cc 13e-92) described in the **AOCS (1993)**.

3.2.2.3- Sensory Evaluation

The sensory evaluation of olive oil was determined by panel test according to the method described in **IOC (2013a).**

3.2.2.4- Acidity

The acidity (as oleic acid %) of oils was determined according to the method outlined in **AOAC (2005)**.

3.2.2.5- Iodine Value (IV)

The iodine value (g of I/100g oil) was also calculated from the fatty acid composition according to **Kyriakidis and Katsiloulis (2000)**.

$$\text{I.V.}_{\text{calculated}} = XC_1 + YC_2 + ZC_3$$

Where, for EVOO: $X=0.93$; $Y=1.35$; $Z=2.62$

for SO: $X=0.95$; $Y=1.60$; $Z= 2.62$

for others: X=1.00 ; Y=1.50 ; Z= 2.62

3.2.2.6- Peroxide Value (PV)

The peroxide value (mequivalent of O_2/kg oil) was determined according to the procedure described in **AOAC (2005)**.

3.2.2.7- Saponification Value

The saponification value (mg of KOH/g oil) was determined according to **AOAC (2005)**.

3.2.2.8- Unsaponifiable Matter

The unsaponifiable matter percentage was determined according to **AOAC (2005).**

3.2.3- Ultraviolet (UV) Spectroscopic Characteristics

Natural conjugated constituents were determined by measuring UV absorption at specific wavelengths in purified solvent.

The sample was dissolved in fresh distilled ethanol (95%) to a known concentration. The absorption was then read at 232 nm for conjugated diene, at 270 nm for conjugated triene in quartz cell with 1 cm path length against a solvent blank in a matching cell using a PyeUnicam double-beam spectrophotometer, model SP1800, as described by **Kates (1972)**, Absorptivity for conjugated constituents was calculated, applying the following relationship: $K = A / bc$

where; A = observed absorbance

b = cell length in cm

c = g test portion / L (final dilution used for K measurement)

The absorption was also read at 266 and 274 nm to calculate ΔK according to **IOOC (2001)** as following: $\Delta K = K_{270} - [\,(K_{266} + K_{274}) / 2\,]$

3.2.4- Oxidative Stability

The oxidative stability of oils was evaluated by the Rancimat method **(Gutiérrez, 1989).** Stability was expressed as induction period

(IP) per hours measured using Rancimat 679 apparatus (Metrohm, Switzerland).

3.2.5- Fatty Acid Composition

The fatty acid composition was released as methyl ester according to **IOOC (2001)** as following:

In a 5 ml screw-top test tube approximately 0.1 g of the oil sample was weighed, 2 ml of heptane was added and shaked well. 0.2 ml of 2N methanolic potassium hydroxide solution was then added, putting on the cap fitted with PTFE-Joint, the cap was tightened and the tube was shaked vigourously for 30 seconds then left to stratify until the upper solution became clear. The upper layer was decanted that contained the methyl esters. The heptane solution was then injected into the gas-liquid chromatography instrument (HP 6890N, Agilent Technologies, USA) equipped with a split/splitless injector (split ratio 1:100) with flame ionization detector (FID) and DB-23 capillary column of 60 m × 0.32 mm ID, 0.25 µm film thickness was filled with 50% cyano-propyl-methyl-poly-siloxane. The injector temperature was 250^{o}C and the detector temperature was 275^{o}C. The temperature of the column was programmed initially at 150^{o}C then allowed to rise at a rate of 10^{o}C/min till reaches 170^{o}C then allowed to rise at a rate of 5^{o}/min till reaches 192^{o}C then holding at this temperature for 4 min. then allowed to rise at a rate of 10^{o}C/min till reaches 220^{o}C then holding at this temperature for 3 min. Nitrogen gas was used as carrier gas with constant flow rate 3.0 ml/min. Hydrogen gas was used as fuel gas with constant rate 3.3 ml/min. and air with constant rate 33 ml/min. The volume injected was 1µl and injected into the column by a Hamilton micro syringe.

The fatty acids present in the sample under investigation were thus identified by comparison of relative retention times (RT) and peak areas which are estimated by computerized process, then these peak areas were used to calculate the fatty acid content expressed as percentage of total fatty acids, applying the following relationship:

Area % Fatty Acid$_X$ = $[A_X / A_T] \times 100$, where;

A_X = area counts of fatty acid methyl esters X

A_T = total area counts for chromatogram

The standard solution composed of standard methyl esters of fatty acids used for identification and matching of samples, it contained the following methyl esters of fatty acids: palmitic (16:0), palmitoleic (16:1), heptadecanoic (17:0), heptadecenoic (17:1), stearic (18:0), oleic (18:1), linoleic (18:2), linolenic (18:3), arachidic (20:0), eicosanoic (20:1), behenic (22:0) and lignoceric (24:0). This standard was obtained from Sigma Chemical Company, USA.

3.2.6- Antioxidant Activity (Free Radical Scavenging Activity) of Phenolic Compounds

The free radical scavenging activities of the oils were measured by 1,1-diphenyl-2-picryl-hydrazil (DPPH) using the method of **Blois (2002).** 0.1mM solution of DPPH in methyl alcohol was prepared and 1mL of this solution was added to 3mL of methanolic phenolic extract of oil. The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Then the absorbance was measured at 517nm in a spectrophotometer. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity.

The capability to scavenge the DPPH* radical was calculated using the following equation:

DPPH* scavenging effect (%) = $[(A_0 - A_1/A_0) \times 100]$

where, A_0 was the absorbance of the control reaction and A_1 the absorbance in the presence of the sample of edible oil.

Results were compared with standard gallic acid in methanol (300ppm).

3.2.7- Chlorophylls and Carotenoids Determinations

Pigment amounts were calculated using the specific extinction values, according to **Minguez-Mosquera *et al.* (1991).**

3.2.7.1- Chlorophyll Compounds

A weight of 7.5 g of edible oil was dissolved in 25 ml of cyclohexane. The extinction coefficients applied were $E_0 = 613$ for pheophytin 'a'. Thus, chlorophyll contents were calculated as follows:

[chlorophyll] mg/kg = $(A_{670} \times 10^6) / (613 \times 100 \times d)$

where A; absorbance , d; spectrophotometer cell thickness (1 cm).

3.2.7.2- Carotenoid Compounds

A weight of 7.5 g of edible oil was dissolved in 25 ml of cyclohexane. The extinction coefficients applied were $E_0 = 672$ for lutein 'a'. Thus, carotenoid contents were calculated as follows:

[carotenoid] mg/kg = $(A_{470} \times 10^6) / (2000 \times 100 \times d)$

where A; absorbance , d; spectrophotometer cell thickness (1 cm).

3.2.8- Colorimetric Determination of Total Phenolic Compounds

The quantitative determination of total phenols in the studied oils was colorimetric assay, following the method described by **Gutfinger (1981)**, with simple modifications. The method is based on the reaction of Folin–Ciocalteau reagent with the functional hydroxy groups of phenolic compounds. Five milliliters of the aqueous-methanolic solution of phenolic compounds extracted from the oil were diluted in 15 ml of ultrapure water, followed by the addition of 1.25 ml of Folin–Ciocalteau reagent and maintained for 3 min. Then, 2.5 ml of sodium hydroxide 6 % (v/v) were added. After 5 min, the reaction mixture was mixed and diluted with ultra pure water to 25 ml.

The absorbance of the solution was measured after 2 h against a blank sample using a PyeUnicam double-beam spectrophotometer, model SP1800 at a wavelength of 725 nm. The calibration curve was constructed using standard solutions of caffeic acid within the range 0–10 mg/l. Results were expressed as milligrams of caffeic acid per kilogram of oil.

3.2.9- Colorimetric Determination of Total Flavonoid Compounds

The total flavonoids (TF) content was evaluated according to **Ivanova *et al.* (2011),** a colorimetric assay with aluminum chloride. 1 ml of the oil was placed in a 10 ml volumetric flask, containing 4 ml of distilled water, followed with addition of 0.3 ml solution of $NaNO_2$ (0.5 g l^{-1}). Solution of 1 g l^{-1} of $AlCl_3$ (0.3 ml) was added 5 min later and after 6 min, 2 ml of NaOH solution (1 mol l^{-1}) was added. The total volume was made up to 10 ml with distilled water and the solution was mixed. The absorbance was measured against the blank (prepared in the same way with distilled water) at 510 nm using a 1 cm optical path. (+)-Catechin was used as a standard for the calibration curve and the TF content was expressed in mg g^{-1} fresh mass as catechin equivalent.

3.2.10- Colorimetric Determination of Total Tocopherol Compounds as α-Tocopherol

Total tocopherols of oils were determined according to the method described by **Wong *et al.* (1988)** as follows: 200 mg of the oil sample were weighed accurately into a 10 ml volumetric flask. 5 ml of toluene were added and the oil taken into solution. 3.5 ml of 2,2'-bipyridine (0.07% w/v in 95% aqueous ethanol) and 0.5 ml of $FeCl_3.6H_2O$ (0.2 w/v in 95% aqueous ethanol) were added in that order. The solution was made up to 10 ml with 95% aqueous ethanol. After standing for one minute the absorption at 520 nm was measured using a blank solution as a reference. Solutions should be protected from strong light during color development. The method was calibrated by preparing standards containing 0.240 µg of pure α-tocopherol in 10 ml of toluene and then analyzing as above.

The concentration of tocopherol in sample was calculated as:

Total tocopherol (ppm) = (A-B) / M.W.

Where, A= absorption of sample in 10 mm cell

B= absorption of blank in 10 mm cell

M= gradient of absorbance vs. weight graph for α-tocopherol calibration; was ca. $8.0x10^{-3}$ absorbance/ μg α-tocopherol.

3.2.11- High Performance Liquid Chromatography (HPLC) Analysis

3.2.11.1- Phenolic Compounds

Phenolic compounds were determined by HPLC according to **Goupy *et al.* (1999)** as follows: 5 g of sample were mixed with methanol and centrifuged at 1000 rpm for 10 min and the suspension was filtered through a 0.2 μm Millipore membrane filter then 1-3 ml were collected in a vial for injection into Agilent 1200 HPLC Liquid Chromatrography System equipped with autosampling injector, solvent degasser, ultraviolet (UV) detector set at 280 nm and quaternary HP pump (series 1050). The column temperature was maintained at 35^{o}C. Gradient separation was carried out with methanol and acetonitrile as a mobile phase at flow rate of 1 ml/min. Phenolic acid standard from Sigma Co. was dissolved in a mobile phase and injected into HPLC. Retention time and peak area were used in the calculation of phenolic compounds concentrations by the data analysis of HP software.

3.2.11.2- Flavonoid Compounds

Flavonoid compounds were determined by HPLC according to **Mattila *et al.* (2000)** employing the same procedure described above in the determination of phenolic compounds with exclusion of setting the ultraviolet (UV) detector at 330 nm and using flavonoid acid standard obtained from Sigma Co. which was dissolved in a mobile phase and injected into HPLC. Retention time and peak area were used in the calculation of flavonoid compounds concentrations by the data analysis of HP software.

3.2.11.3- Tocopherol Compounds

Tocopherol compounds were determined by HPLC according to **Carpenter (1979)** following also the above same procedure used in the determination of phenolic and flavonoid compounds except setting the ultraviolet (UV) detector at 295 nm and using tocopherol standard obtained from Sigma Co. that was dissolved in a mobile phase and injected into HPLC. Retention time and peak area were used in the calculation of tocopherol compounds concentrations by the data analysis of HP software.

3.2.12- Sterols Composition

The composition of sterols was determined according to the method (COI/ T.20/ Doc. No 30/Rev. 1) outlined in **IOC (2013c).**

Preparation of the basic thin layer chromatography plates was carried out by immersing or dipping the silica gel plates (about 4 cm) in the 0.2 M ethanolic potassium hydroxide solution for 10seconds, then allowed to dry in a fume cupboard for two hours and finally placed in an oven at 100ºC for one hour, then removed from the oven and kept in a calcium chloride desiccator until required for use. Hexane/ethyl ether mixture was placed into the development chamber, to a depth of approximately 1 cm. The chamber was closed with the appropriate cover and left thus for at least half an hour, in a cool place, so that liquid-vapor equilibrium is established strips of filter paper dipping into the eluent may be placed on the internal surfaces of the chamber. This reduces developing time by approximately one-third and brings about more uniform and regular elution of the components.

An approximately 5% solution of the unsaponifiable in ethyl acetate was prepared and 0.3 ml of the solution on a narrow and uniform streak on the lower end (2 cm) of the chromatographic plate was deposed. In line with the streak, 2 to 3 µl of the material reference solution were placed, so that the sterol band can be identified after developing.

The plate was sprayed lightly and uniformly with the 2,7-dichlorofluorescein solution and then left to dry. When the plate is observed under ultraviolet light, the sterols bands can be identified through being aligned with the spots obtained from the reference solution.

By using a metal spatula, the silica gel of the marked area was scraped off. The finely comminuted material was removed into the filter funnel. 10 ml of hot ethyl acetate were added and mixed carefully with the metal spatula and filtered under vacuum, the filtrate was collected in the conical flask attached to the filter funnel. The residue in the flask was washed three times with ethyl ether (approximately 10 ml each time), the filtrate was collected in the same flask attached to the funnel, then evaporated to a volume of 4 to 5 ml, the residual solution was transferred to the previously weighed 10 ml test tube, evaporated to dryness by mild heating, in a gentle flow of nitrogen, make up again using a few drops of acetone, and evaporated again to dryness. The residue content in the test tube consisted of the sterol fraction.

For preparation of the trimethylsilyl ethers, the silylation reagent at ratio of 50 μl for every milligram of sterols fraction were added, avoiding any uptake of moisture, was Stoppered and shaked carefully (without overturning) until the compounds were completely dissolved and left to stand for at least 15 minutes at ambient temperature then centrifuge for a few minutes. The clear solution was ready for gas chromatographic analysis. The sterols were eluted in the following order: cholesterol, campesterol, stigmasterol, ß-sistosterol, Δ5-avenasterol, Δ7-avenasterol.

3.2.13- Fourier Transform Infrared (FTIR) Spectral Analysis

FTIR spectral analysis was carried out according to the method outlined by **Guillén and Cabo (1999)** as follows:

A number of 4 sets of fresh oils; extra virgin olive, moringa, apricot kernel and sunflower oils were exposed to FTIR analysis using a Jasco Spectrum FTIR Plus 460 Spectrophotometer (Japan) equipped with a deuterated tri-glycine sulphate (DTGS) detector to obtain FTIR spectra.

The standard detector in routine FTIR instruments is the pyroelectric DTGS (deuterated tri-glycine sulfate) detector, whose response in the MIR range is wavelength independent. The MIR spectral range extends from 4000 to 400 cm^{-1}. The detector operates at ambient temperature and shows good linearity across the whole transmittance scale. The DTGS detector responds to signal frequencies of up to several thousand Hz, hence the time needed to scan one spectrum at a resolution of 4 cm^{-1} is of the order of 1 second. A small quantity (one drop of the sample) was pressed between two well-polished KBr disks (liquid cell) creating a thin film.

Each sample undergoes 64 scans which were accumulated in one measurement to acquire a sufficient signal-to-noise ratio. Samples were scanned between 4000 and 400 cm^{-1} with a nominal resolution of 4 cm^{-1} and the data interval was 1cm^{-1}. Time required for acquiring a complete sample spectrum was 30 second.

Fourier transformed infrared spectra of these oils were recorded taking into account the close relationships found previously between the frequency data of some specific bands and the composition of the oil samples, frequency data of all samples were collected and used in equations that relate frequency and composition.

3.2.14- Nuclear Magnetic Resonance (NMR) Spectral Analysis

3.2.14.1- ^{1}H-NMR Spectral Analysis

^{1}H-NMR spectral analysis was carried out according to the method outlined by **Guillén and Ruiz (2001)** as follows:

Oils samples under study were dissolved in deuterated dimethyl sulphoxide (DMSO-d6) solvent for ^{1}H-NMR Determination. ^{1}H-NMR spectra were recorded on a Varian Gemini 300 BB NMR Spectrometer (USA) with Oxford 300 MHz NMR Magnet, Gemini 2000 Console and 5 mm probe, located at Main Chemical Laboratory in Ministry of Defense, Egypt. Observing ^{1}H at 300 MHz at temperature of 25°C, ^{1}H-NMR spectra were acquired using spectral width of 8000 Hz, relaxation delay of 1 sec, 64 repetitions, acquisition time of 1.998 sec and with a total time of 3 min, 37 sec. Chemical shifts were expressed in δ units (ppm).

3.2.14.2- ^{13}C-NMR Spectral Analysis

^{13}C-NMR spectral analysis was carried out according to the method outlined by **Guillén and Ruiz (2001)** as follows:

Oil samples under study were dissolved in deuterated dimethyl sulphoxide (DMSO-d6) solvent for ^{13}C-NMR Determination. The four oil samples were also dissolved in deuterated chloroform ($CDCl_3$) to show the solvent effect and the difference between DMSO-d6 and $CDCl_3$ as solvents. ^{13}C-NMR spectra were recorded on a Varian Gemini 300 BB NMR Spectrometer (USA) with Oxford 300 MHz NMR Magnet, Gemini 2000 Console and 5 mm probe, located at Main Chemical Laboratory in Ministry of Defense, Egypt. Observing ^{13}C at 300 MHz at temperature of 25^{o}C. Chemical shifts were expressed in δ units (ppm).

4- RESULTS AND DISCUSSION

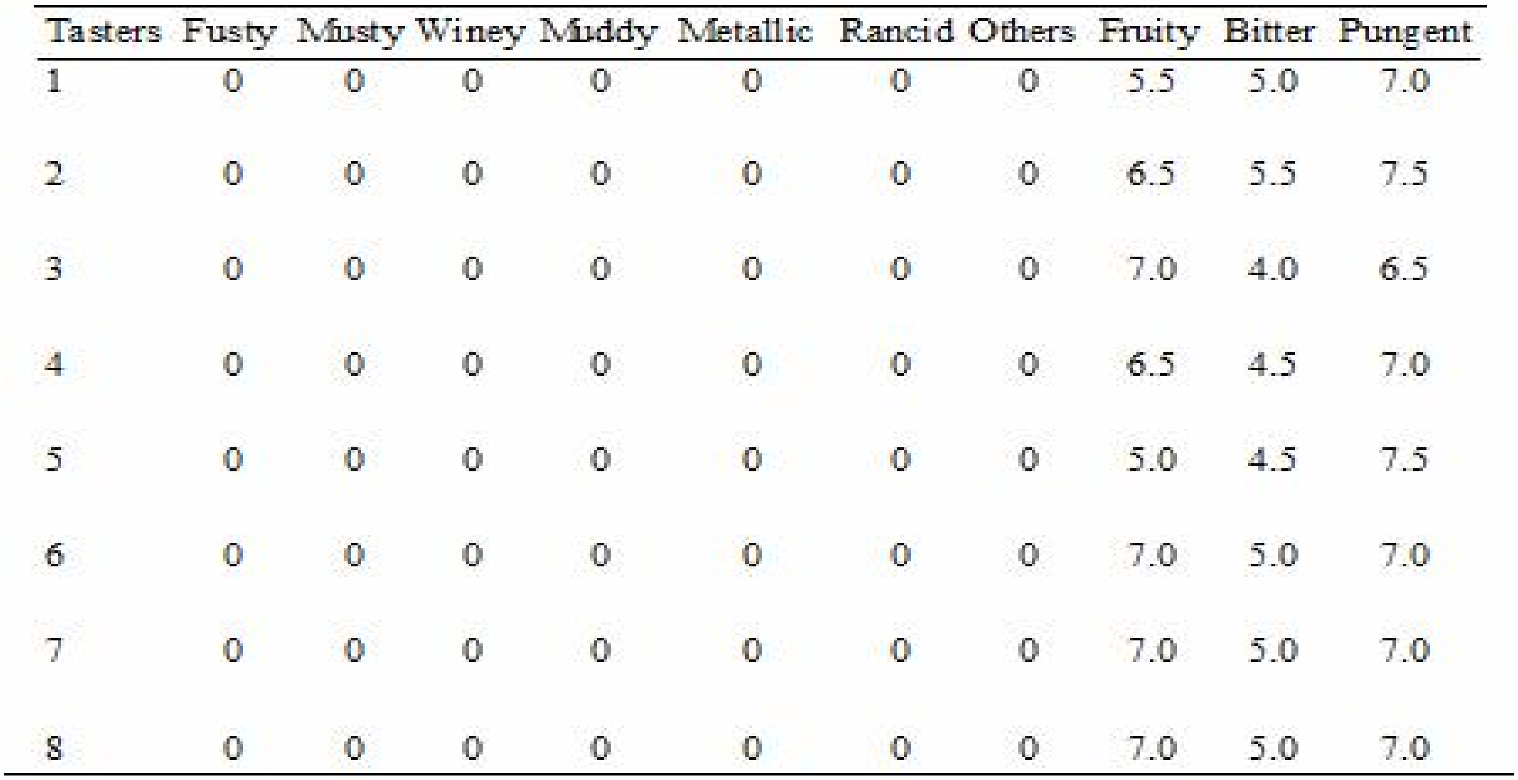
The current work was planned to evaluate the stability of four extracted edible oils namely extra virgin olive, moringa, apricot kernel and sunflower oils. These oils were analyzed to identify the relation between their composition and stability with great stress to investigate what compounds are responsible to their stability. The oils were exposed to their physical and chemical characteristics determination and fatty acid composition, as well as, the identification of sterols, tocopherols, phenols and flavonoids composition. The evaluation of oxidative stability by Rancimat method, and the activity of the antioxidants of oils were also carried out.

In addition, the oils were conducted to FTIR and NMR spectral analysis to investigate useful analytical methods which reflect the stability of oils.

Before analysis, to be sure enough that the extracted olive oil was extra virgin, the oil was submitted to sensory evaluation by eight members well trained. The panelists were asked to score the oil for its fruity, bitter and pungent besides, any defects occurred in the oil such as fusty, musty, winey, muddy, metallic, rancid and others, each from ten.

One of the most important aspects of olive oil classification and value determination is sensory evaluation. Human sensory evaluation is much more accurate (100 times) for olive oil than laboratory equipment. Aroma and taste are very complex and cannot be determined in the laboratory. The first and primary objective in sensory evaluation for olive oil is to determine if oils contain one or more of the defects that commonly occur in oils. Olive oil should have a fruity olive flavor that is characteristic of the variety making up the oil. There should be no vinegary or fermented odor or flavor. The oil should also not be rancid or possess any other off flavor that is essentially not of the olive. The second objective of oil-sensory evaluation is to describe the positive characteristics of the oil in relation to its intensity of olive-fruity character.

Table (1) Sensory evaluation of olive oil



Tasters	Fusty	Musty	Winey	Muddy	Metallic	Rancid	Others	Fruity	Bitter	Pungent
1	0	0	0	0	0	0	0	5.5	5.0	7.0
2	0	0	0	0	0	0	0	6.5	5.5	7.5
3	0	0	0	0	0	0	0	7.0	4.0	6.5
4	0	0	0	0	0	0	0	6.5	4.5	7.0
5	0	0	0	0	0	0	0	5.0	4.5	7.5
6	0	0	0	0	0	0	0	7.0	5.0	7.0
7	0	0	0	0	0	0	0	7.0	5.0	7.0
8	0	0	0	0	0	0	0	7.0	5.0	7.0

Bitterness and pungency are often present in olive oils, especially when newly made. They are not defects and will mellow as the oils age. The numerical sensory values for the grade (extra virgin) come from a rating of the oil by a qualified taste panel.

Results obtained in **Table (1)** classified olive oil as extra virgin olive oil (EVOO) revealed no any defect was present in the oil. However, the oil fruitiness, bitterness and pungency received a scores ranging between 5.5-7.0, 4.5-5.5 and 6.5-7.5 with mean values of 6.4, 4.5 and 7.1, respectively indicating that the studied olive oil was classified as extra virgin olive oil according to the International Olive Council **(IOC, 2013a).**

4.1- Physical and Chemical Characteristics of Oils

4.1.1- Refractive Index

The refractive index (RI) is an important physical characteristic of oils because of the ease and speed with which it can be determined precisely, the small quantity of sample needed, and its relationship to structure. It generally gives a good idea about the degree of unsaturation of oil, as well as its correlation with the iodine value. Refractive index increases with unsaturation and correlates with iodine values. Lower refractive index could be related to the higher free fatty acid content. It has been reported that the refractive indices of oils increase on auto-oxidation and can be used to evaluate the rancidity of oils. The increase of refractive index with auto-oxidation is possibly attributable to conjugation known to precede hydroperoxide formation in the secondary stage, and polymerization of partially oxidized fats and oils in the tertiary state of oxidation since both conjugation and polymerization are reported to result in increased refractive indices of oils and fats. Oils of different types exhibited different refractive indices. This indicates that refractive index can be used in the preliminary identification of fats and oils.

Table (2) Refractive index (at 25°C) and color index of the studied oils

Oil	Refractive index	Color index		
		Yellow	Red	Blue
EVOO	1.4696	35	2.2	3.2
MO	1.4621	35	3.6	0.5
AKO	1.4647	35	0.6	0.0
SO	1.4739	35	0.2	0.0

From **Table (2)**, it could be seen that the refractive index measured at 25°C of moringa oil was the smallest one (1.4621) comparing to the values of 1.4647, 1.4696 and 1.4739 for apricot kernel oil, extra virgin olive oil and sunflower oil, respectively. These variations in refractive indices of the corresponding oils under investigation were attributed to their structure, chain length and the differences in fatty acid composition of these oils containing different profiles, especially linoleic acid (C18:2) content as shown later in **Table (5)**.

Considering the order of the increment of the refractive indices values in relation to linoleic acid (**Tables 2** and **5**), moringa oil had the least refractive index value of 1.4621 with the lowest percentage of linoleic acid (0.75%), followed by apricot kernel oil with the refractive index value of 1.4647 corresponding to 23.13% linoleic acid content, then comes the extra virgin olive oil having refractive index value of 1.4696 corresponding to 11.04% of linoleic acid being lesser than its content in the apricot kernel oil, at least sunflower oil having the highest refractive index value of 1.4739 among the aforementioned oils corresponding to 52.20% of linoleic acid.

It was obviously clear that there was a direct proportional relationship between percentages of linoleic acid ($C_{18:2}$) and the refractive indices. The more the increase in refractive index, the highly the degree of unsaturation, especially dienoic fatty acid in the oil. This phenomenon was true except for apricot kernel oil which came in the second order after moringa oil preceding the extra virgin olive oil in its linoleic acid percentage. The higher refractive index of extra virgin olive oil with corresponding lower linoleic acid percentage than of apricot kernel oil may be due to the difference in structure between the oils, and the present of other unsaturated compounds in the extra virgin olive oil other than acyl chains of unsaturated fatty acids that reflected its higher refractive index.

The obtained refractive indices of the studied oils agreed with **Codex standard for olive oils (2013); Atabani *et al.* (2013a, 2013b); Ogunsina *et al.* (2014); Sharma *et al.* (2012); Bisht *et al.* (2013); Nehdi *et al.* (2013); Codex standard for named vegetable oils (2013).**

4.1.2- Color Index

The most common colored materials in crude oils are naturally occurring pigments such as red from carotenoids and green from chlorophyll. These colors are generally removed substantially in the bleaching and deodorization processes. In this study, Lovibond was used for the measurement of oils color using three colored scales (yellow, red and blue). The yellow scale was fixed at 35. From the obtained results in **Table (2)**, the tested oils differed markedly in their red color, where moringa oil had the highest value (3.6) followed by extra virgin olive oil (2.2), then apricot kernel oil (0.6) and sunflower oil (0.2). These findings coincided with the carotenoids content in oils under investigation (**Table 10**), where moringa oil had the highest content of carotenoids (4.210 mg/kg) followed by extra virgin olive oil (3.210 mg/kg), apricot kernel oil (0.155 mg/kg) and sunflower oil (0.045 mg/kg). The blue color indices appeared for only extra virgin olive and moringa oils, with the highest value for extra virgin olive oil (3.2) coinciding with the highest chlorophyll content (1.778 mg/kg) comparable to moringa oil which had a low blue color index (0.5) corresponding to its chlorophyll content of (0.538 mg/kg) which was much lower than that of extra virgin olive oil as shown later in **Table (10).**

It was noticed that the extra virgin olive oil characterized by a pale red with dark blue greenish color. However, moringa oil had a dark reddish yellow with blue tinge color. Among apricot kernel oil, it had a popular yellow reddish tinge color, while the crude sunflower oil was bright yellow in color.

The results obtained from the color index parameter may be used to make preliminary identification of the quality and freshness state of crude oil because in the case of oil refining and bleaching, much of pigments as carotenes and chlorophyll will be removed and will not give any values in the red and blue indices which together are responsible for pigments.

4.1.3- Acidity

Acidity is a measure of the amount of free fatty acids present in oil due to both hydrolysis of its triglycerides and oxidation of double bonds of the unsaturated acyl chains which produced free fatty acids with low molecular weight. It has been frequently used as an important parameter to monitor the quality of oils and to show the case of hydrolysis and oxidation which are induced in the oil.

The acidity of extra virgin olive, moringa, apricot kernel and sunflower oils expressed as oleic acid percentage are presented in **Table (3).** It was clear from the obtained data that the amount of free fatty acids formed by hydrolysis and oxidation were generally too small to affect the quality of the oils. It was 0.18, 0.91, 0.69 and 0.07% for extra virgin olive, moringa, apricot kernel and sunflower oils, respectively. The observed low acidity indicated that the oils may have a long shelf life. Nevertheless, because the determination of free fatty acids by titration does not differentiate between acids formed by oxidation and those formed by hydrolysis, it is a poor measure of oil deterioration.

Table (3) Chemical parameters of the studied oils

Oil	Acidity %	Saponification value	Unsaponifiable matter %	I.V. (Calculated)	P.V.
EVOO	0.18	186.10	2.19	81.46	1.01
MO	0.91	194.70	1.90	73.65	0.00
AKO	0.69	193.50	1.80	105.37	0.00
SO	0.07	183.00	1.35	116.96	0.50

From **Tables (2** and **3**), it was noticed that there exists inversely proportional relations between refractive indices and acidity values for oils under investigations. The least acidity (0.07%) was recorded for sunflower oil which had the greatest refractive index (1.4739). While, moringa oil showed the highest acidity (0.91%) and its refractive index was the least one (1.4621). The order of increasing refractive indices and decreasing acidity was: MO < AKO < EVOO < SO.

4.1.4- Saponification Value

Saponification value, a measure of alkali reactive groups in oils, was used to predict the type of glycerides in oil. Glycerides containing short-chain fatty acids have higher saponification values than those with longer chain fatty acids. The saponification value, along with the iodine value determination, were useful screening tests both for quality control and for characterizing types of oils. It represents the number of milligrams of potassium hydroxide required to saponify 1g of oil. It is a measure of the average molecular weight (or chain length) of all the fatty acids present. It allows for comparison of the average fatty acid chain length. The long chain fatty acids found in oils have a low saponification value because they have a relatively fewer number of carboxylic functional groups per unit mass of the oil as compared to short chain fatty acids.

The data presented in **Table (3)** gave an idea about the nature of the chain length of the studied oils. It was clear that moringa oil had slightly higher saponification value (194.70), compared to other oils, which reflect the shorter acyl chain of fatty acids, followed by apricot kernel oil having a close saponification value to that of moringa oil (193.50), then extra virgin olive oil with a value of 186.10, and at least sunflower oil having the lowest saponification value (183.00).

4.1.5- Unsaponifiable matter

Table (3) also showed the percentage of unsaponifiable matter of the studied oils. It could be noticed that the extra virgin olive oil characterized by the highest percentage of unsaponifiable matter (2.19%) followed by moringa oil (1.90), then apricot kernel oil having a closed unsaponifiable matter percentage (1.80%). The lowest percentage was found in sunflower oil (1.35%). It was appeared that all the oils under investigation had a high unsaponifiable matter values, especially the extra virgin olive and moringa oils indicating their high stability followed by apricot kernel oil and sunflower oil.

From **Tables (2, 3),** it was noticed that there was an inversely proportional relation between saponification values and the refractive index values for oils under investigations. The increasing order of saponification value of oils was: SO < EVOO < AKO < MO. The least saponification value recorded for sunflower oil was correlated with the highest refractive index value (1.4739). While, moringa oil which showed the greatest saponification value (194.7) had the least refractive index one (1.4621).

4.1.6- Iodine Value

The iodine value is defined as a number of grams from iodine which absorbed by 100 grams of oil or fat. It represents one of the parameters that could differentiate between the qualities of oils and is normally used to give an idea about oils constituents. The iodine value is used to indicate the degree of unsaturation of oil fatty acids or for measuring the number of double bonds present in the molecule of oil. The higher the iodine value, the higher the unsaturation of the oil. Iodine value analyses are very accurate and provide nearly theoretical values, except in the case of conjugated double bonds or when the double bond is near a carboxyl group.

The results given in **Table (3)** indicated that sunflower oil was highly unsaturated; it had iodine value of 116.96. However, the apricot kernel oil was less unsaturated followed by extra virgin olive oil, their iodine values were 105.37 and 81.46, respectively. Moringa oil had the least iodine value being 37.65. The low iodine values of extra virgin olive and moringa oils indicate that these oils had a high resistance to oxidation.

It was noticed that the least iodine value (73.65) recorded for moringa oil corresponded the least refractive index (1.4621) and highest saponification value (194.70). Whereas, the highest iodine value (116.96) recorded for sunflower oil corresponded the highest refractive index (1.4739) and least saponification value (183.00). From **Tables (2, 3),** it was clear that there was a directly proportional relation between iodine values and refractive indices from one hand, and inversely proportional relationships with saponification values from the other hand, for oils under investigations. Thus, it could arrange the oils descendingly depending on their iodine values and refractive index as follows: SO > EVOO > AKO > MO; and according to their saponification values as follows: MO > AKO > EVOO > SO.

4.1.7- Peroxide Value

Detection of peroxide value gives the initial evidence of rancidity in unsaturated fats and oils. It gives a measure of the extent to which an oil sample has undergone primary oxidation. Oils with a high degree of unsaturation are most susceptible to autoxidation. The best test for autoxidation is the determination of the peroxide value.

The primary products of lipid oxidation are hydroperoxides which are generally referred to as peroxides. Therefore, it seems reasonable to determine the concentration of peroxides as a measure of the extent of oxidation of a fat or oil. However, this theory is limited due to the transitory nature of the peroxides which are intermediate products in the formation route of carbonyl and hydroxyl compounds that leads to deterioration of fats and oils which form off-flavors and off-odors. Thus, the peroxide value indicates the case of oxidative rancidity of any oil and the quantity of oxidized substances, normally hydroperoxides that liberate iodine from potassium iodide under specified condition. It is expressed as milliequivalents of active oxygen per kilogram of oil.

As expected in **Table (3)**, the extra virgin olive and sunflower oils showed a low peroxide values being 1.005 and 0.5, respectively. Whereas, moringa and apricot kernel oils did not show any peroxide values. It was clear that that obtained values were much lower than permitted maximum peroxide level stipulated by the **Codex standards**, which not over than 10 for vegetable oils. The low peroxide values of the studied oils indicated that these oils were not subjected to any serious rancidity before extraction.

The values obtained of the chemical properties of the tested oils are in accordance with **Bulut and Yılmaz (2010); Sharma *et al.* (2012); Bisht *et al.* (2013); Codex standard for named vegetable oils (2013); Codex standard for olive oils (2013); IOC trade standard applying to olive oils and olive pomace oils (2013a); Nehdi *et al.* (2013); Farhoosh and Yazdi (2014); Ogunsina *et al.* (2014); Rohman *et al.* (2014).**

4.1.8- Ultraviolet (UV) Spectral Analysis

The spectrophotometric measurement is one of the physical methods which is particularly useful in composition studies on fats and oils and in the analysis and identification of fatty materials. It had been employed for studying and following chemical reactions of fatty materials, especially isomerization, polymerization, oxidation and the determination of rancidity in oils. Unsaturated fatty materials absorb light in the UV region of the spectrum; this region ranged between 100 and 400 nm. In the case of non-conjugated and saturated materials, the absorption is weak and cannot be used for analytical purposes. When linoleic and linolenic acids or more highly unsaturated fatty acids are oxidized to form hydroperoxides, the double bonds in the oils become conjugated. The linoleic and linolenic acids are isomerized to conjugated dienoic and trienoic acids, respectively; this isomerization is applied to direct quantitative determination of these acids content.

The hydroperoxides and the conjugated diene which may result from its decomposition show a strong absorption band at about 232 nm. However, the low absorbency at 266 nm indicates the absence of the secondary products of oxidation. While conjugated triene and the secondary oxidation products, particularly diketones, show an absorption band at 270 nm.

Absorbencies at 232, 270, 266, 274 nm and ΔK of extra virgin olive, moringa, apricot kernel and sunflower oils are listed in **Table (4).** It could be noticed that the major absorption peak was at 232 nm, followed by the absorbencies at the other wavelengths which showed small differences among them.

The obtained results indicated that moringa oil had the lowest absorption band at 232 nm being 1.018 followed by that of extra virgin olive oil (1.411) then that of apricot kernel oil (1.415). However, sunflower oil gave rise to intense at 232 nm having relatively the highest absorption of 2.717.

Table (4) Spectral Absorbencies at 232, 270, 266, 274 nm and ΔK of the studied oils

Oil	Abs_{232} (Diene)	Abs_{270} (Triene)	Abs_{266}	Abs_{274}	ΔK
EVOO	1.411	0.010	0.041	0.029	-0.025
MO	1.018	0.074	0.079	0.059	0.005
AKO	1.415	0.131	0.135	0.116	0.005
SO	2.717	1.437	1.420	1.234	0.110

The obtained lower values of moringa, extra virgin olive and apricot kernel oils could be due to their low concentration of linoleate, and also to the highest antioxidant effectiveness of these oils. While, the higher UV absorbance at 232 nm might be attributed to the conjugated dienoic acids already incorporated in this oil, and it could be also due to its higher polyunsaturated fatty acid linoleic (52.20%, **Table 5**) than that of other oils which contained lower percentage of this acid being 23.13, 11.04 and 0.75% for apricot kernel oil, extra virgin olive oil, and moringa oil, respectively. When the polyunsaturated linoleate is oxidized to form hydroperoxides, a shift in the double bonds occurs lead to absorption at 232 nm.

It was noticed that the UV absorbencies at 232 nm were correlated positively to linoleic acid concentration in the oils.

Concerning the UV absorption at 270 nm, extra virgin olive oil showed a very small value of 0.010 which agrees with those reported by **IOC trade standard applying to olive oils and olive pomace oils (2013a); Codex standard for olive oils (2013).** However, the values of K_{270} for pure moringa, apricot kernel and sunflower oils were considerably higher than that of extra virgin olive oil being 0.074, 0.131 and 1.437, respectively.

The small value in absorbency at 270 nm of extra virgin olive oil might be further contributed to its low concentration of linoleate coupled with surplus oxidative stability of this oil, and subsequently low relative rates of formation of ketonic and aldehydic oxidative products, as well as low formation of conjugated trienes. While, the more absorption at 270 nm caused by the other studied oils which exceeded that of extra virgin olive oil, especially sunflower oil, since moringa oil still had small values in spite having higher values than that for extra virgin olive oil, might be attributed to their higher oxidative degradation of the polyunsaturated linoleate present in these oils at higher concentrations resulting in the increase formation of conjugated trienoic and ketodienes. The excess UV absorbance at 270 nm could be also explained by the higher amount of activated methylene groups in polyunsaturated fatty acids in these oils, especially sunflower oil than in extra virgin olive oil which are oxidized to form secondary oxidative products.

The ΔK was calculated using the UV absorbencies at 270, 266 and 274 nm as follows: $\Delta K = [\text{Abs}_{270} - (\text{Abs}_{266} + \text{Abs}_{274})/2]$

ΔK is useful for readily classifying olive oil quality according to its value. ΔK for extra virgin olive oil ≤ 0.01 as stipulated by **IOC trade standard applying to olive oils and olive pomace oils (2013a); Codex standard for olive oils (2013); Rohman *et al.* (2014).**

ΔK values for moringa, apricot kernel and sunflower oils were 0.005, 0.005 and 0.110, respectively, higher than that of extra virgin olive oil. Nevertheless, all the obtained ΔK values were very small indicating no oxidative rancidity occurred in the oils. Additionally, all the obtained spectral absorbencies at all the measured wavelengths for all the tested oils are considered as small values.

Results obtained in this study are close to those reported by **Latif and Anwar (2009); Bulut and Yılmaz (2010); Nadeem *et al.* (2014).**

4.1.9 – Fatty Acid Composition

Fatty acids are the basic building blocks of most lipids. Usually, the analysis of fatty acids from oils is performed by gas-chromatography (GC) and involves a pre-dramatization of the combined fatty acids into less polar and more volatile compounds, so that they can be eluted at reasonable temperatures, without thermal decomposition or molecular rearrangement along the GC column. The most common procedure consists in the hydrolysis and methylation of the combined fatty acids to form their respective methyl esters (FAMEs).

The fatty acids composition of extra virgin olive, moringa, apricot kernel and sunflower oils were determined using GC, and the obtained results are presented in **Tables (5)** along with useful relations from the fatty acid composition of oils listed in **Table (6).**

The major fatty acids are those C16 and C18 saturated fatty acids (SFA) mainly palmitic and stearic acids and C18 mono- or polyunsaturated fatty acids (PUFA) mostly oleic, linoleic and linolenic acids. As expected in **Table (5)**, all the studied oils had elevated amounts of total unsaturated fatty acids ranged between 76.86% in moringa oil to 94.40% in apricot kernel oil. The most predominant unsaturated fatty acids were oleic and linoleic acids. From the composition point of view, extra virgin olive, moringa, and apricot kernel oils had a high content of monounsaturated oleic acid, its percentage was closed among each others being 68.33, 71.98 and 69-56%, respectively. However, sunflower oil contained the lesser concentration of oleic acid (34.23%), while it had superior content of the polyunsaturated linoleic acid, its percentage reached 52.20%. On the other hand, linoleic acid was found in minor proportions in moringa oil (0.75%), and in low percentages in extra virgin olive oil (11.04%) and apricot kernel oil (23.13%).

Table (5) Fatty acid composition % of the studied oils

Fatty Acid	EVOO	MO	AKO	SO
C 12:0	*ND	*ND	*ND	*ND
C 14:0	*ND	*ND	*ND	*ND
C 16:0	14.62	6.84	5.23	7.83
C 16:1	0.83	1.82	0.94	0.13
C 17:0	0.04	0.10	0.04	0.05
C 17:1	0.07	0.05	0.14	0.03
C 18:0	2.12	5.04	0.00	3.32
C 18:1	69.34	71.98	69.56	34.23
C 18:2	11.04	0.75	23.13	52.20
C 18:3	0.80	0.21	0.42	0.35
C 20:0	0.50	3.40	0.21	0.41
C 20:1	0.49	2.05	0.21	0.26
C 22:0	0.14	6.60	0.12	0.76
C 22:1	*ND	*ND	*ND	*ND
C 24:0	0.02	1.17	*ND	0.42
C 24:1	*ND	*ND	*ND	*ND
Σ SFA	17.43	23.14	5.60	12.79
Σ USFA	82.57	76.86	94.40	87.21

*ND: Not Detected

Table (6) Relationships obtained from fatty acid composition of the studied oils

Oil	EVOO	MO	AKO	SO
Σ SFA / Σ USFA	0.21	0.30	0.06	0.15
Σ MUFA	70.72	75.90	70.84	34.66
Σ PUFA	11.84	0.96	23.56	52.55
Σ PUFA / Σ MUFA	0.17	0.01	0.33	1.52
Σ MUFA / Σ PUFA	5.90	79.03	3.01	0.66
C 16:0 / C 18:2	1.32	9.13	0.23	0.15
C 18:1 / C 18:2	6.28	96.11	3.01	0.66
C 18:2 / C 18:3	13.85	3.54	54.71	149.71
$\frac{\text{C 18:2 + C 18:3}}{\text{C 18:1}}$	0.17	0.01	0.34	1.54

Concerning the saturated fatty acids, their total ranged between 5.60% in apricot kernel oil and 23.14% in moringa oil. Palmitic acid was found to be the most prevalent acid in all the studied oils, its percentage was 14.62, 6.84, 5.23 and 7.83% for extra virgin olive, moringa, apricot kernel and sunflower oils, respectively, followed by stearic acid, its concentration was 2.12, 5.04, 0.00 and 3.32% in the above respective oils.

In conclusion, it was observed from the results of the GC analysis of the tested oils, that moringa, extra virgin olive and apricot kernel oils which characterized with their high contents of monounsaturated fatty acid oleic and low contents in the polyunsaturated linoleic acid, makes these oils more stable.

The obtained data are in accordance with those reported by **Alpaslan and Hayta (2006); Abdulkarim *et al.* (2007); Anwar *et al.* (2007); Manzoor *et al.* (2007); Merrill *et al.* (2008); Latif and Anwar (2009); Bulut and Yılmaz (2010); Durmaz *et al.* (2010); Gharachorloo *et al.* (2010); Codex standard for named vegetable oils (2013); IOC trade standard applying to olive oils and olive pomace oils (2013a); Codex standard for olive oils (2013); Nehdi *et al.* (2013); Farhoosh and Yazdi (2014); Nadeem *et al.* (2014): Ogunsina *et al.* (2014); Rohman *et al.* (2014).**

Because the global proportion of unsaturated fatty acids is somewhat similar in these oils, this result was justified by a higher PUFA/MUFA ratio in sunflower oil. This may show a predictable behavior towards oxidation which correlates directly with PUFA/MUFA ratio. As shown in **Table (6)**, the order of decreasing PUFA/MUFA ratio started by the highest value recorded for sunflower oil (1.52) of the lowest stability, followed by apricot kernel oil (0.33), then extra virgin olive oil (0.17), and at least comes moringa oil (0.01) with the highest stability and the lowest PUFA/MUFA ratio. This ratio may be listed as MUFA/PUFA, the inverse PUFA/MUFA ratio, where instead of assigning the highest value for sunflower oil, the value was inversed to be the lowest one as in **Table (6).** These results agreed with **Roman *et al.* (2013).**

It was noticed that the relative ratios of palmitic acid over linoleic acid ($C_{16:0}/C_{18:2}$) and oleic acid over linoleic acid ($C_{18:1}/C_{18:2}$) were correlated with the relative ratio of monounsaturated fatty acids over polyunsaturated fatty acids (Σ MUFA / Σ PUFA) which were a helpful index for distinguishing oils under study. In addition, the ratio of linoleic acid over linolenic acid ($C_{18:2}/C_{18:3}$) was also helpful to distinguish the oil. Sunflower oil with the lowest stability has the highest ratio (149.71) followed by apricot kernel oil (54.71) then extra virgin olive oil (13.85) and at last comes moringa oil with the lowest ratio (3.54) indicating its highest stability.

Moreover, a very useful relationship was the summation of linoleic and linolenic acids over oleic acid ($[C_{18:2} + C_{18:3}] / C_{18:1}$), where sunflower oil recorded the highest value (1.54) indicating its lowest stability, followed by apricot kernel oil (0.34), the extra virgin olive oil (0.17), then the lowest value that recorded for moringa oil (0.01) indicating its highest stability. These results agreed with **Ceci and Carelli (2010).**

4.1.10- Chlorophylls and Carotenoids of Oils

Chlorophyll and carotenoid compounds play an important role in edible oils. They interfere in the oxidative stability acting as antioxidants in the dark and acting as prooxidants when exposed to light. Besides that, these compounds are responsible for the yellow green pigmentation of edible oils, increasing consumer’s acceptability.

Chlorophylls are non-polar photosensitizers located mainly in oil particles. In their natural environment, they are fairly stable. However, as a consequence of cellular destruction and the mixing of cellular content during oil extraction, many modifications in the pigments take place. During the extraction process, chlorophylls are transformed into their respective magnesium-free derivatives.

Carotenoids are important biological pigments that play vital role in cell membrane stability, cellular differentiation, growth control, photo-oxidative protection, vision process, photosynthesis and in nutrition as pro-vitamin A. The carotenoids are one of the most important groups of natural pigments in fruits and vegetables. Several epidemiological studies have found their antioxidant properties through a link between high-carotenoid-content vegetable intake and a lower risk of chronic degenerative diseases such as cancer or cardiovascular diseases. Carotenoids are 40-carbon isoprenoids or tetraterpenes with varying structural characteristics. They are easily susceptible to attack by electrophilic reagents, because of the highly reactive, electron-rich system of conjugated double bonds on their long polyene chain, forming thereby stable radicals and enhancing therefore their antioxidants properties. The antioxidant efficiency of β-carotene depends on the balance between radical trapping and auto-oxidation.

Table (7) Chlorophylls and carotenoids (mg/kg) and the chlorophyll / carotenoids ratio of the studied oils

Oil	Chlorophyll	Carotenoids	Chlorophyll / Carotenoids
EVOO	2.78	3.21	0.87
MO	0.54	4.81	0.11
AKO	0.11	0.16	0.74
SO	0.21	0.05	4.71

Table (7) lists chlorophylls and carotenoids content (mg/kg) and (chlorophyll / carotenoids) ratio of the tested oils. It was clearly noticed that EVOO had the highest content of chlorophyll (2.78 mg/kg) followed by MO (0.54 mg/kg) then SO (0.21 mg/kg) and AKO (0.11 mg/kg). The highest content of chlorophylls in olive oil is mainly responsible for its color that varying from yellow-green to greenish gold. These pigments are also important in olive oil stability their antioxidant nature in the dark and pre-oxidant activity in the light.

On the other hand, MO had the highest content of carotenoids (4.81 mg/kg) followed by EVOO (3.21 mg/kg) then AKO (0.16 mg/kg) and SO (0.05 mg/kg). The behavior of these pigments is similar to that of chlorophylls. It was noticed that carotenoids content reflect the stability of the tested oils, their concentrations correlated positively with the oils oxidative stability measured by Rancimat method (**Table 9**), where the oils were descendingly arranged according to their stability as follows: MO > EVOO > AKO > SO.

From **Table (7)**, a good relationship was obtained between chlorophylls / carotenoids ratios and oils stabilities, where the highest ratio recorded for SO (4.71) indicated its lowest stability, whereas, MO recorded the lowest ratio (0.11) indicating its highest stability. Among AKO it had a ratio of 0.74 following MO, and then EVOO (0.87). It was noticed a great difference in the ratio between MO and SO, but the difference range is very narrow between AKO and EVOO because EVOO has the greatest chlorophyll content among all the oils under investigation which may be reflected in its greater ratio (chlorophyll / carotenoids) than AKO, although EVOO had higher stability than AKO by the common Rancimat method. In this respect, because EVOO contained the highest concentration of chlorophylls, these compounds arranged descendingly the oils according to their contents as follows: EVOO > MO > AKO > SO. This arrangement was in accordance with the order of the oils stability by carotenoids and Rancimat except MO comes after EVOO in their stability. The results obtained agreed with **Malheiro *et al.* (2009); Dimakou and Oreopoulou (2012).**

4.1.11- Tocopherols, flavonoids and polyphenols

Tocopherols are naturally present in edible oils and play an important role in their resistance to oxidation processes mainly due to their antioxidant capacity. Tocopherols are important analytical parameters because of their vitamin and nutritional properties and their role in protecting edible oils from free radicals.

Total tocopherols, total flavonoids and total polyphenols content of oils are shown in **Table (8).** The obtained data revealed that EVOO had the highest respective values of total tocopherols and total polyphenols being 193.94; 1312.73 mg/kg followed by MO (70.60; 462.75 mg/kg), then AKO (68.43; 419.50 mg/kg) and SO (364.57; 48.43 mg/kg). However, EVOO recorded also the highest total flavonoids value (411.50 mg/kg) followed by AKO (41.84 mg/kg) then SO (27.40 mg/kg), whereas, MO recorded the lowest total flavonoids value (25.91 mg/kg) among all the tested oils.

It was noticed that the total tocopherols content along with the total polyphenols arranged the oils descendingly according to their concentrations as follows: EVOO > MO > AKO > SO. This arrangement was similar to the order of oils stability obtained by chlorophyll compounds.

Table (8) Tocopherols, flavonoids and polyphenols (mg/kg) of the studied oils

Oil	Tocopherols	Flavonoids	Polyphenols
EVOO	193.94	411.50	1312.73
MO	70.60	25.91	462.75
AKO	68.43	41.84	419.50
SO	88.43	27.40	364.57

From the aforementioned results, it could be said that the high stability of EVOO is mainly due to its relatively low degree of polyunsaturated fatty acids (11.84 %) and to its antioxidant activity of the unsaponifiable components, tocopherols, flavonoids and phenolic compounds which also showed synergistic effect with each others. The obtained results agree with other findings reported by **Alonso-Salces *et al.* (2011); Gharby *et al.*, (2012); Malheiro *et al.* (2013).**

4.1.12- Oxidative Stability of Oils by Rancimat

Oxidative stability is a very important parameter for evaluating the quality of oils once it gives a good perception and estimation of the susceptibility to oxidation process. It is defined as the time necessary for an oil to start showing symptoms of rancidity after accelerated oxidation of the unsaturated fatty acids. The Rancimat test is an official method that is recognized internationally as well as by numerous countries such as the United States, Japan and Switzerland. The Rancimat test measures resistance to accelerated oxidation This test was therefore performed to evaluate the oxidative stability of the oils and gave the induction period

(IP), expressed in hours, where, the length of time the more, the oil resisted oxidative stress. The IP value is reported on a PC directly connected to the Rancimat apparatus.

Table (9) Oxidative stability by Rancimat method (20 $l^{-1}h$, 100^oC) of the studied oils with and without their natural polyphenols

Oil	IP	IP without polyphenols
EVOO	59. 11	31.91
MO	190.00	171.20
AKO	26.60	25.60
SO	7.45	5.86

The results of oxidative stability, in terms of measurement of induction periods (Rancimat, 20 l h^{-1}, 100^oC) of the tested oils (EVOO, MO, AKO and SO) before and after removal of the natural polyphenols present in oils are shown in **Table (9)**. The data showed that moringa oil had the highest induction period being 190.00 hr indicating superior resistance to oxidation, followed by extra virgin olive oil (59.11 hr), then apricot kernel oil (26.00 hr). However, sunflower oil recorded only 7.45 hr. The oxidative stability of moringa oil was about triple than that of extra virgin olive oil; 7.3 fold than that of apricot kernel oil and more than twenty five fold than that of sunflower oil which had the least induction period. The highest resistance of moringa oil to oxidation followed by extra virgin olive oil was due to less linoleate, higher percentage of oleate and unsaponifiable matter. It also attributed to the higher antioxidant content of tocopherols, flavonoids, sterols and phenolics substances as well as chlorophyll and carotenoids which presented in high levels in the oils.

It was noticed that the apricot kernel oil had a middle induction period having relatively high stability against oxidation due to its higher percentage in oleic acid, but it had higher linoleate and lower antioxidant content compared to moringa and extra virgin olive oils. It could be observed that the higher oleic acid oils, moringa, extra virgin olive and apricot kernel had relatively high stability against oxidation comparable to sunflower oil.

The lowest induction period of sunflower oil was attributed to its lower antioxidant content and higher polyunsaturated fatty acid linoleic (52.2%) and the high degree of unsaturation which allowed the oil to oxidize easily. As, it is known that linoleic acid oxidize 50 times faster than oleic acid.

Considering the measurement of oxidative stability by the Rancimat method for oils without polyphenols (**Table 9**) to study the effect of polyphenols on the oxidative stability of oils, it was observed an overall decrease in the IP of the oils after removing their polyphenols beginning from MO which recorded the highest IP (171.20 hr), followed by EVOO (31.91 hr), then AKO (25.60 hr) and least comes SO (7.45 hr) as an oil with last stability. The differences between the values before and after the removal of polyphenols reflect the antioxidant activity of these components in the oils.

It was noticed that the highest drop took place in the induction period of the extra virgin olive oil which declined from 59.11 to 37.91 hr by a percentage of 46.02%, followed by sunflower oil having an induction period of 7.45 hr and descended to 5.86 hr being by 21.34%, then moringa oil which its induction period fall down from 190.00 to 171.20 hr, by 9.89%. While, a small breakdown was happened in the induction period of apricot kernel oil from 26.00 to 25.60 hr which was by only 3.76% although its content of polyphenols was higher than that of sunflower oil and closed to that of moringa oil.

These obtained results indicated that the activity of polyphenols differed between the studied oils, which may be due to the differences in phenolic composition and concentration, and also may be attributed to the synergistic effect of its components.

It could be also observed that the polyphenols were a main antioxidant compounds in EVOO which contained the highest value of 1312.73 mg/kg compared to MO (462.75 mg/kg), AKO (419.50 mg/kg) and SO (364.57 mg/kg) as shown in **Table (8)**. This can be explained by the fact that the oils having a higher content of total polyphenols are acknowledged to be of higher stability due to higher antioxidants manifested by these polyphenols in oils. Also, besides having quite a high content of these antioxidant compounds, it has a low content of polyunsaturated fatty acids and a high MUFA/PUFA ratio.

EVOO showed a high oxidative stability due to its high content of polyphenols. However, MO which had the highest stability, included another factors other than polyphenols because it had a lower content of these components than EVOO and also its IP decreased only by 9.89% after removal of polyphenols compared with EVOO whose IP decreased by 46.02% which was appeared that the polyphenols approached half of its IP. In other words, polyphenols are responsible for about half of the stability of EVOO.

It could be observed that the phenol fraction had a more powerful action against radicals. Both IP values and tests are able to scavenge a free radical (DPPH) depend on the phenolic pool, even though the Rancimat value takes into account the whole oil moiety. The Rancimat test only gives a total estimation of the antioxidant potential of the oil, without information on the possible contribution of single compounds and their positive or negative interactions. Then, oxidative stability is not dependant on a single parameter, but is rather affected by the fatty acid composition and a complex pool of antioxidants and prooxidants.

This coincides with **Gharby *et al.* (2012).** Hence, a positive correlation was recorded between the polyphenols content of the oils and their oxidative stability.

4.1.13- Antioxidant Activity of Phenolic Compounds

Phenolic compounds are structurally characterized by the presence of hydroxyl groups bound to aromatic rings. These hydroxyl groups are able to neutralize the free radicals by means of the donation of a hydrogen radical, leading to the formation of the chemically stable phenoxide radical, interrupting the progress of the auto-oxidation reaction.

Table (10) Antioxidant activity of phenolic extracts of the studied oils by DPPH Method

Oil	Radical Scavenging Capacity %
EVOO	92.91
MO	48.82
AKO	45.18
SO	25.18
Gallic Acid (300 ppm)	90.22

The tested oils (EVOO, MO, AKO, SO) reported a capacity to scavenge the free radicals of DPPH between 25.18% and 92.91% with standard reference of gallic acid (300 ppm), whose radical scavenging capacity was 90.22% as shown in **Table (10)**. EVOO had the highest value of radical scavenging capacity (92.91%), followed by MO (48.82%), AKO (45.12%), then SO (25.18%).

4.1.14- Sterol composition

The composition of sterol fraction of the studied oils was analyzed by GLC, and the results are shown in **Table (11)**. The obtained data revealed that the most predominant sterol in all the tested oils was *β*-sitosterol, its highest percentage was noticed in extra virgin olive oil which accounted 88.08% followed by apricot kernel oil (87.80%), sunflower oil (68.09%) them moringa oil with the least value of *β*-sitosterol being 51.07%. However, moringa oil contained the highest percentage of stigmasterol among the other oils, it was the second prevalent one after *β*-sitosterol, its concentration was 17.87% in moringa oil, followed by sunflower oil (10.01%), while both extra virgin olive and apricot kernel oils contained a few proportions of stigmasterol being 1.00 and 0.90%, respectively.

Table (11) Sterol composition % of the studied oils

Sterols	Oils sterol composition %			
	EVOO	MO	AKO	SO
Cholesterol	0.019	0.013	0.00	0.10
Campesterol	3.92	15.20	4.20	9.31
Stigmasterol	1.00	17.87	0.90	10.01
β-Sitosterol	88.08	51.07	87.80	68.09
Δ^5- Avenasterol	6.23	13.48	*ND	1.10
Δ^7- Avenasterol	0.30	1.13	*ND	4.11
Other	*ND	*ND	5.00	6.00

*ND: Not Detected

Moringa oil contained also the highest percentage of campesterol which was in the third order after stigmasterol in this oil; its percentage was 15.20%, followed by sunflower oil (9.31%), extra virgin olive oil (3.92%), and then apricot kernel oil (4.20%).

On the other hand, moringa oil contained a higher percentage of Δ^5- avenasterol (13.48%) more than twice its content in extra virgin olive oil (6.23%), whereas this sterol was 1.10% in sunflower oil and was not found in apricot kernel oil. Among Δ^7- avenasterol, its percentage was relatively higher in sunflower oil (4.11%) followed by moringa and extra virgin olive oils containing very small concentrations of this sterol (1.13 and 0.30%, respectively), while it was absent in apricot kernel oil.

Concerning the sterol fraction cholesterol, it was found in extra virgin olive and moringa and sunflower oils in trace percentages of 0.019, 0.013 and 0.10%, respectively and nothing else.

It could be observed that the antioxidant activity of some sterols, especially β-sitosterol which is one of the most abundant component of olive oil unsaponifiable sterol fraction besides Δ^5- avenasterol may be mainly responsible for the oil oxidative stability. β-sitosterol is seems to be also a main factor in apricot kernel oil and sunflower oxidative stability. While, the main antioxidant components in moringa oil unsaponifiable sterol fraction were β-sitosterol, stigmasterol, campesterol and Δ^5- avenasterol which they may be in totally responsible for its oxidative stability.

4.1.15- Tocopherol Composition

Tocopherols are known as the powerful lipid radical scavengers. Their antioxidant effect, however, strongly depends on various parameters such as their concentration, degree of unsaturation of the lipid substrate and the presence of other antioxidants.

Table (12) showed the tocopherols composition of the studied oils expressed as mg/kg oil. It was noticed that the extra virgin olive oil contained the highest amount of α-tocopherol being 145.58 mg/kg, followed by sunflower oil (115.20 mg/kg), moringa oil (68.20 mg/kg), then at least apricot kernel oil (5.00 mg/kg). However, the α-tocopherol was presented in its highest concentration in apricot kernel oil (93.50 mg/kg), followed by moringa oil (29.01 mg/kg), extra virgin olive oil, then sunflower oil (0.02 mg/kg). Concerning δ- tocopherol, moringa oil characterized by the highest concentration of this fraction which reached 98.16 mg/kg, while, apricot kernel, extra virgin olive and sunflower oils contained a rarely quantities of this tocopherol being 1.50, 0.50 and 0.001, respectively.

These results indicated that the most abundant tocopherol in both extra virgin olive and sunflower oils was the α-tocopherol; and in apricot kernel oil was the γ- tocopherol, whereas, in moringa oil the most predominant one was the δ- tocopherol with a considerable amounts of α-tocopherol and γ- tocopherol.

Table (12) Tocopherol composition (mg/kg) of the studied oils

Oil	Tocopherol composition		
	α-tocopherol	γ- tocopherol	δ- tocopherol
EVOO	145.58	7.80	0.50
MO	68.20	29.01	98.16
AKO	5.00	93.50	1.50
SO	115.20	0.02	0.001

Thus, tocopherols presented in high concentrations in moringa oil that mostly was δ- tocopherol which its antioxidant activity exceeds that of α-tocopherol and γ- tocopherol, and was contribution to the highest resistance to oxidation, and making clear the long induction period of this oil among others.

Considering extra virgin olive oil it was expected to be less stable than moringa oil and had a shorter induction period than moringa oil. In this respect, **Baldioli *et al.* (1996)**, showed that the contribution of α-tocopherol to extra virgin olive oil oxidative stability, although its impact, evaluated by Rancimat method, was smaller compared to those of phenolic, fatty acid composition and total chlorophylls.

From the obtained data, it was observed no correlation between α, γ, or δ-tocopherols concentration and the induction period measured by Rancimat method of the studied oils. It means that the rearrangement of oils according to the concentration of tocopherols was not correlated with their induction periods. This may be commentary because at high temperature, the tocopherols are unstable, especially α-tocopherol, so they contribute little to defining the induction period value.

4.1.16- Phenol Composition

The most important phenolic compounds in the studied oils are shown in **Table (13).**

Table (13) Phenol composition (mg/kg) of the studied oils

Phenols	Phenols concentration present in oil (mg/kg)			
	EVOO	MO	AKO	SO
Syringic acid	4.47	*ND	*ND	*ND
Gallic acid	0.04	0.02	0.17	*ND
Pyrogallol	62.71	1.43	3.81	0.73
3- Hydroxy tyrosol	174.12	0.07	3.06	*ND
4-Amino benzoic acid	1.38	*ND	*ND	0.01
Protocatechuic acid	3.60	0.03	0.08	0.01
Catechin	4.88	0.01	0.01	0.01
Cholrogenic acid	4.92	0.13	0.03	0.05
Catechol	87.18	0.05	0.10	0.03
Epicatechin	1.42	0.18	0.22	0.18
Caffein	1.92	0.02	0.04	0.02
p-OH benzoic acid	1.80	0.37	0.20	0.07
Caffeic acid	1.86	0.01	0.02	0.02
Vanillic acid	1.29	0.14	0.41	0.02
p-Coumaric	1.51	*ND	*ND	*ND
Ferulic acid	1.30	0.02	0.03	0.02
Iso-ferulic acid	0.48	*ND	*ND	*ND
Reversterol	3.33	*ND	0.70	0.47
Ellagic acid	13.42	1.44	0.57	0.28
Oleuropein	68.90	0.48	1.65	0.69
α- Coumaric acid	0.67	*ND	*ND	*ND
Benzoic acid	39.84	0.55	2.58	0.82
Salicylic acid	9.20	0.71	0.49	0.46
Coumarin	11.36	0.15	0.11	0.15
3,4,5 Met. cinnamic acid	9.54	*ND	*ND	*ND
Cinnamic acid	2.32	0.11	0.11	0.03

*ND: Not Detected

It was clearly noticed that moringa oil, apricot kernel oil and sunflower oil contained a very small quantities less than 1 ppm for each of all the estimated phenolic compounds, except the phenols; pyrogallol, 3-hydroxytyrosol, oleuropein and benzoic acid were presented in a few proportions of 3.81, 3.06, 1.65 and 2.58 ppm, respectively in apricot kernel oil. Pyrogallol and ellagic acid among all the phenols, were found also in moringa oil at rarely concentration of 1.43 and 1.44 ppm, respectively.

EVOO contains a considerable quantity of phenolic compounds which pass from the fruit to the oil during extraction. These fine compounds are natural antioxidants. Ortho-diphenols such as hydroxytyrosol, caffeic acid and oleuropein are considered to be the most powerful antioxidants. They protect olive oil from oxidation, make it more stable during storage and contribute to oil flavor and aroma; they lend the oil a bitter taste and pungent sensation. Their content in the oil likewise depends on several factors, namely variety and olive fruit ripeness, conditions of extraction and storage conditions. Phenolic content is an important parameter in the evaluation of the extra virgin olive oil quality. A high total phenolic content appears to be beneficial for the shelf life of the oil where a good correlation of stability and total phenol content was observed.

It was observed the presence of a high number of phenolic compounds in EVOO, notably: 3-hydroxytyrosol (3, 4-DHPEA) (174.12 ppm), catechol (87.18 ppm), oleuropein (68.90 ppm), pyrogallol (62.71 ppm), benzoic acid (39.84 ppm), ellagic acid (13.42 ppm), coumarin (11.36 ppm), 3,4,5 met. cinnamic acid (9.54 ppm), salicylic acid (9.20 ppm), syringic acid (4.47 ppm), reversterol (3.33 ppm) and cinnamic acid (2.32 ppm).

Vanillic (1.29 ppm) and ferulic (1.30 ppm) acids are hindered phenols, since the -OCH_3 group ortho or meta to the hydroxyl group suppresses antioxidant activity. This steric hindrance is likely responsible for the relative ineffectiveness of vanillic and ferulic acids. In addition, the caffeic acid, having two -OH groups at adjacent positions, acts as a chelator for most of the metal ions that act as pro-oxidants and that may catalyze the reaction even if present in trace amounts.

Among all phenols, hydroxytyrosol, caffeic acid, coumaric acid and *p*-hydroxybenzoic acid exhibit the greatest effect on the sensory characteristics of olive oil.

The obtained results agreed with those obtained by **Dabbou *et al.*, (2010); Kesen *et al.* (2013).**

4.1.17- Flavonoid Composition

Flavonoids are plant pigments that are synthesized from phenylalanine and generally display marvelous colors in the flowering parts of plants. Flavonoids comprise a large group of polyphenolic compounds that are characterized by a Benzo-y-pyrone structure, which are ubiquitous in vegetables and fruits. Besides their relevance in plants, they are important for human health because of their high pharmacological activities as radical scavengers. Flavonoids possess a broad spectrum of chemical and biological activities including radical scavenging properties. Flavonoids of oils were determined and summarized in **Table (14).**

It was observed that EVOO had the highest content of flavonoids. The main flavonoids present in EVOO were listed descendingly according to their abundance in EVOO as follows: kampferol, hespertin, narengin, hisperdin, quercetrin, quercetin, 7-hydroxy flavone, apigenin, luteolin, rutin, narengenin and rosmarenic. Luteolin and apigenin are main flavonoids present in olive oils, which originate from their corresponding glucosides present in the drupe. Concentrations of luteolin and apigenin in EVOO were 0.68 and 0.77 ppm, respectively. Whereas, MO and AKO contained flavonoids in much lower contents than had EVOO with the absence of luteolin in MO but it appeared in AKO, and the absence of apigenin in both MO and AKO which was present in EVOO only. Also, SO had a considerably very low content of flavonoids with the absence of luteolin and apigenin. The obtained results agreed with those reported by **Ocakoglu *et al.* (2009).**

Table (14) Flavonoid composition (mg/kg) of the studied oils

Flavonoid	Flavonoid concentration present in oil (mg/kg)			
	EVOO	MO	AKO	SO
Narengin	2.85	0.37	0.53	0.11
Rutin	0.65	0.13	0.17	0.02
Hisperdin	2.59	0.54	0.11	0.08
Rosmarenic	0.25	0.01	*ND	*ND
Quercetrin	2.02	0.15	0.38	0.04
Quercetin	1.53	0.02	0.04	0.03
Luteolin	0.68	*ND	0.02	*ND
Narengenin	0.59	0.04	0.03	*ND
Kampferol	6.03	0.19	0.29	0.17
Hespertin	4.51	0.26	0.15	0.02
Apigenin	0.77	*ND	*ND	*ND
7-Hydroxy Flavone	1.02	0.04	0.06	0.07

*ND: Not Detected

It could be concluded that the studied oils varied greatly in their oxidative stability, which depends on many intrinsic factors such as fatty acid composition and natural levels of pro/antioxidants. These natural antioxidants, despite their low concentrations, prevent oil oxidation. In addition to the individual role of each antioxidant compound to monitor the oils stability, also the effective synergism of all the antioxidants present such as phenols, α-tocopherol and pigments may be contribute in such monitoring.

To the high content of α-, γ- and δ-tocopherol of MO that mostly was δ-tocopherol, which its oxidative stability exceeds that of α- and γ-tocopherol, it could be attributed the higher resistance to oxidation more than EVOO. EVOO contained higher α-tocopherol than MO and a rarely quantity of δ-tocopherol, in addition, it contained linoleic (11.04%) and linolenic (0.80%) acids which they were in much lower quantity in MO (0.75 and 0.21%, respectively) and which were more easily undergone oxidation and degradation than oleic acid. The long induction period of MO might be explained also by the presence of Δ^5-avenasterol. However, EVOO, which had a shorter induction period, had a higher content of that sterol. Furthermore, the higher oxidative stability of MO over EVOO should be attributed to other constituents of non-glyceride fraction of the oil, which possess antioxidant properties.

4.2- Evaluation of Oxidative Stability of Edible Oils using Fourier Transform Infra Red (FTIR) Spectroscopy

The IR spectrum contains the entire information about the molecular structure of the investigated sample. A more chemical approach is the evaluation of characteristic or group frequencies. Some chemical groups exhibit very characteristic bands regardless of the kind of molecule in which they are included. The group frequency approach is very useful for structural analysis. From the frequencies and intensities of spectral bands it is possible to predict what kind of chemical groups are present in the molecule, how they are connected to other groups and finally the structure of the molecule under investigation. The most convenient tool for identification of molecules from their vibrational spectrum is spectral databases. The matching process is very much accelerated by computerized search programs. If an exact match cannot be found, these programs usually list the reference compounds that numerically match the unknown spectrum very closely. Every chemical compound has its own characteristic IR spectrum. The main problem is the assignment of experimental spectral bands. In addition to fundamental vibration bands, very often so-called combination and overtone bands are present. Fermi resonance can cause intensity changes and frequency shifts of the bands involved. Intermolecular interactions (such as hydrogen bonding) can cause additional bands. Furthermore, the influences of solvents, temperature and pressure have to be considered.

Before discussion the obtained spectrum, it was serious to look after the classification of FTIR according to **Gauglitz and Vo-Dinh (2003)**, it may be classified into 3 regions. The first region from 4000 to 1400 cm^{-1}; this region comprises stretching vibrations involving movements of light atoms (molar mass below 20 g mol^{-1}). O–H and N–H stretching bands are located in the region from 3700 to 2500 cm^{-1}. These groups very often participate in the formation of hydrogen bond complexes. The formation of such complexes results in red shifted and very broad spectral bands.

C–H stretching bands are found in the 3300-2800 cm^{-1} spectral range. Bands in the 2700-1850 cm^{-1} spectral region usually belong to C≡C, C≡N, N≡N or some groups containing hydrogen and a heavier atom (S−H, P−H and Si−H). The 1950-1450 cm^{-1} region exhibits IR absorption from a wide variety of double-bonded chemical groups, in particular C=O. Conjugation, ring size, hydrogen bonding, steric and electronic effects often result in significant shifts in absorption frequencies.

The second region from 1400 to 900 cm^{-1}; this is called the fingerprint region. Many chemical groups with single bonds have group frequencies in this region. These vibrations usually couple very strongly, i.e. particular bands in this region can hardly be attributed to a single chemical bond or group. On the other hand, bands caused by complex interacting vibrations constitute a unique fingerprint for each compound. If two spectra exhibit identical fingerprint patterns in this region, the corresponding samples are generally considered to be identical.

The third region from 900 to 400 cm^{-1}; some characteristic bands of aromatics occur in this region. These bands are due to aromatic C–H out-of-plane bending vibrations. The absence of absorption bands in the 900 to 650 cm^{-1} region usually indicates the lack of aromatic rings in the molecule under investigation. Some organic molecules containing halogen atoms can also contribute in this region.

Figures (1-5) show the FTIR spectra of the fresh studied oils: extra virgin olive, moringa, apricot kernel, sunflower and oxidized olive oils, respectively, giving the relation between wavenumber (cm^{-1}) of FTIR spectral region (4000-400 cm^{-1}) versus the transmittance percent (%T). **Tables (15-19)** show FTIR spectral data of the above respective oils, along with assignments of the FTIR signals to the main components of the oils.

It was easily deduced from **Figure (1)** and **Table (15)** that the FTIR spectra of extra virgin olive oil were dominated by peaks around 3470, 3004, 2924, 2854, 2732, 2678, 2378, 2306, 2030, 1746, 1653, 1460, 1373, 1236, 1163, 1118, 1098, 1031 and 722 cm^{-1}.

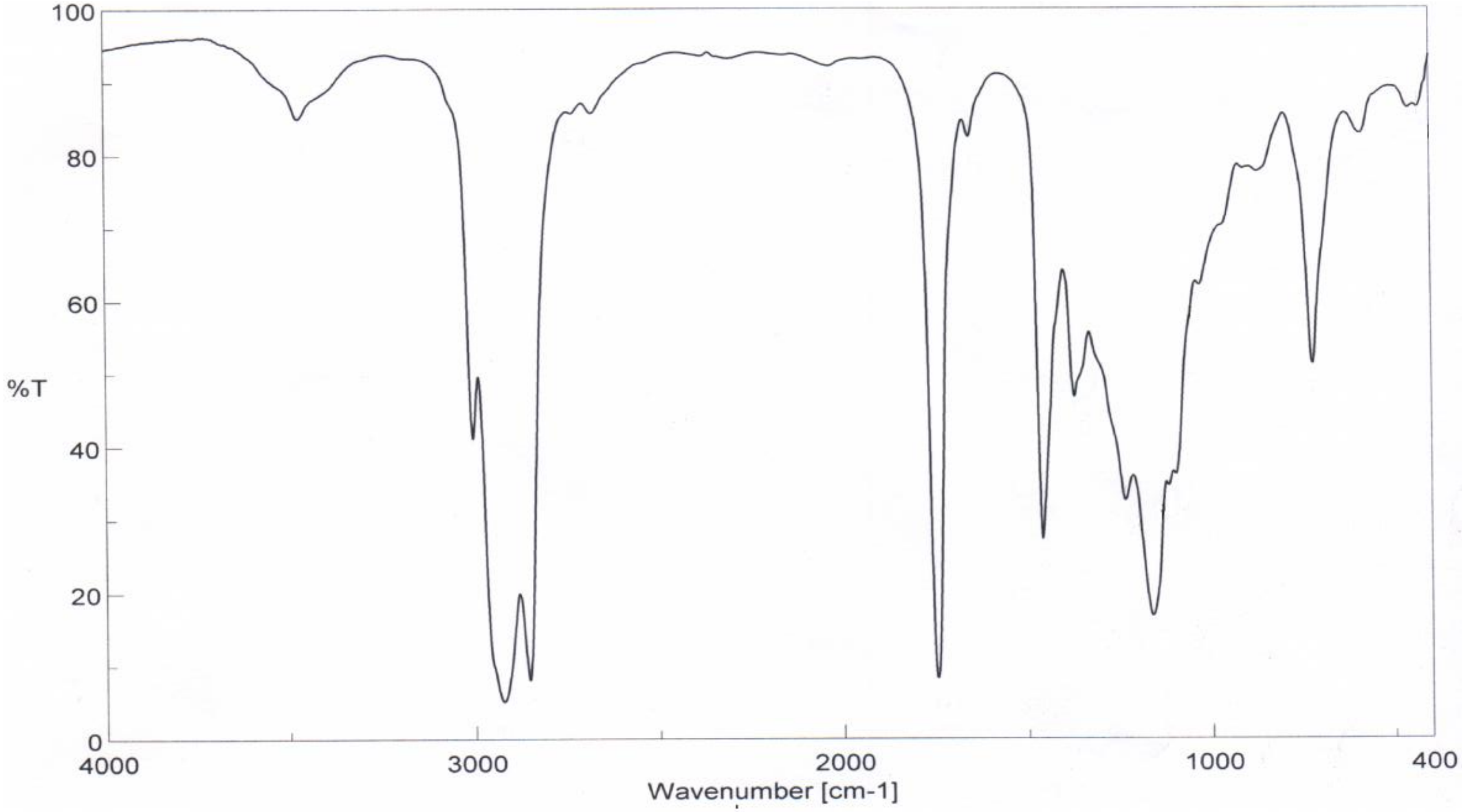


Figure (1) FTIR transmittance spectrum of fresh EVOO

Table (15) FTIR spectral data and assignments of the FTIR signals of the main components of fresh EVOO

FTIR spectral data of fresh EVOO		**Assignments of the FTIR signals**
cm^{-1}	**Intensity %**	
3470.28	84.9656	Overtone of glyceride ester C=O
3004.55	41.1342	=CH (olefinic group of USFA)
2924.52	5.15742	C–H stretching (asymmetry)
2854.13	8.08297	C–H stretching (symmetry)
2732.64	85.6821	Intramolecular hydrogen bonded-OH
2678.64	85.718	C=O Fermi resonance
2378.76	93.5382	O-C=O stretching (asymmetry)
1746.23	8.27813	C=O stretching
1653.66	82.343	C=C stretching (*cis*)
1460.81	27.2903	C–H bending (scissoring)
1373.07	46.7211	C–H bending
1236.15	32.5847	C–H bending
1163.83	16.7683	C–O stretching and C–H bending
1118.51	34.5927	CO ester groups
1098.26	36.1165	C–H bending
1031.73	62.0355	C–H bending
870.703	77.558	C–C stretching
722.211	51.1663	C–H bending (rocking)

Absorbencies at 2924 and 2854 cm$^{−1}$ were due to bands arising from CH_2 stretching vibrations; asymmetric and symmetric, respectively. The main peak at 1746 cm$^{−1}$ was aroused from C=O stretching vibrations; the bands at 1460 and 1373 cm$^{−1}$ were aroused from CH_2 and CH_3 scissoring vibration, while those at 1236, 1163, 1118, 1098 were associated with C–O stretching vibration. Both the frequencies at 1118 and 1163 cm^{-1} have been proved to be related to the amount of saturated acyl groups. The absence of 962 cm^{-1} peak indicated the absence of *trans*–CH=CH– group from the fatty acids. These results agree with those reported by **Bertran *et al.* (1999); Yang and Irudayaraj (2001); Sinelli *et al.* (2007); Gurdeniz and Ozen (2009); Al-Degs *et al.* (2011); Navarra *et al.* (2011).**

Similarly, it was intelligible from **Figure (2)** and **Table (16)** that the FTIR spectra of moringa oil were predominant in peaks around 3471, 3003, 2924, 2854, 2733, 2678, 2319, 2155, 2030, 1745, 1654, 1460, 1373, 1236, 1162, 1117, 1030, 871 and 722 cm^{-1}. The absorption peaks at 2924 and 2854 cm$^{−1}$ were due to bands originated from CH_2 stretching vibrations, asymmetric and symmetric, respectively. The major peak at 1745 cm$^{−1}$ was excited from C=O stretching vibrations; the bands at 1460 and 1373 cm$^{−1}$ were inflamed from CH_2 and CH_3 scissoring vibration, while those bands located at 1236, 1162, 1117 cm^{-1} were associated with C–O stretching vibration. Both the frequencies at 1117 and 1162 cm^{-1} were related to the amount of saturated acyl groups.

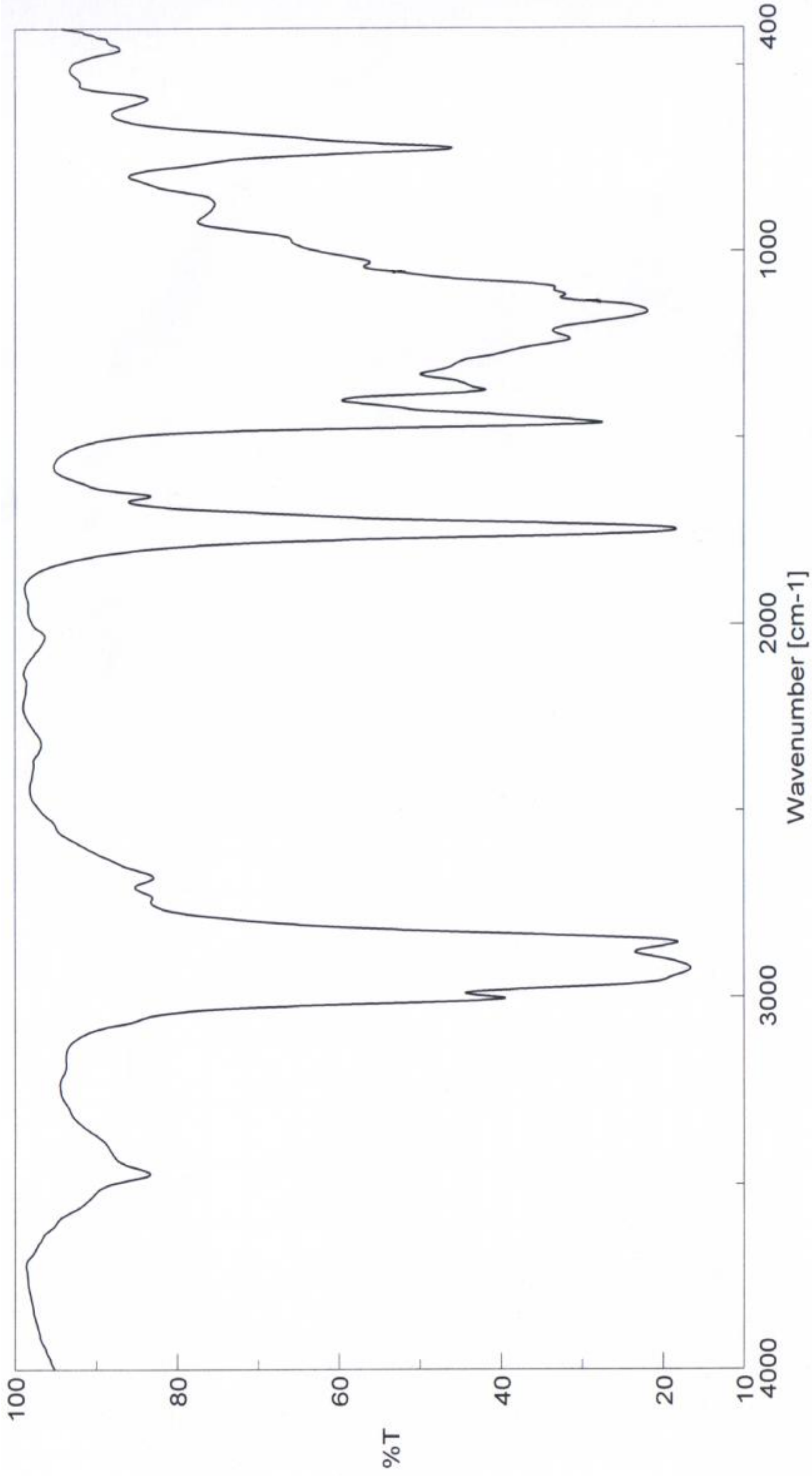


Figure (2) FTIR transmittance spectrum of fresh MO

Table (16) FTIR spectral data and assignments of the FTIR signals of the main components of fresh MO

FTIR spectral data of fresh MO		**Assignments of the FTIR signals**
cm^{-1}	**Intensity %**	
3471.24	83.2993	Overtone of glyceride ester C=O
3003.59	39.4357	=CH (olefinic group of USFA)
2924.52	16.5768	C–H stretching (asymmetry)
2854.13	18.1319	C–H stretching (symmetry)
2733.6	82.9933	Intramolecular hydrogen bonded-OH
2678.64	82.8497	C=O Fermi resonance
1745.26	18.3263	C=O stretching
1654.62	83.1733	C=C stretching (*cis*)
1460.81	27.473	C–H bending (scissoring)
1373.07	41.861	C–H bending
1236.15	31.4455	C–H bending
1162.87	21.9025	C–O stretching and C–H bending
1117.55	31.9718	CO ester groups
1030.77	56.1103	C–H bending
871.667	75.2416	C–C stretching
722.211	46.0406	C–H bending (rocking)

It was easy to find out from **Figure (3)** and **Table (17)** that the FTIR spectra of apricot kernel oil were spread over peaks around 3469, 3005, 2923, 2854, 2678, 2337, 2156, 2031, 1743, 1652, 1459, 1374, 1236, 1163, 1029, 913, 873 and 721 cm^{-1}. Absorbencies at 2923 and 2854 cm^{-1} were due to bands spring from CH_2 stretching vibrations, asymmetric and symmetric, respectively. The major peak at 1743 cm^{-1} was derived from C=O stretching vibrations, while the bands at 1459 and 1374 cm^{-1} were aroused from CH_2 and CH_3 scissoring vibration, while those bands located at 1236, 1163 cm^{-1} were associated with C–O stretching vibration. A small peak around 913 cm^{-1} was assigned to HC=CH, (*cis*), bending out-of-plane.

Also, it is deducible from **Figure (4)** and **Table (18)** that the FTIR spectra of sunflower oil were prevalent in peaks around 3470, 3006, 2924, 2856, 2677, 2334, 2159, 2031, 1936, 1745, 1653, 1457, 1372, 1235, 1163, 914 and 722 cm^{-1}. The absorbencies at 2924 and 2856 cm^{-1} were due to bands preceded from CH_2 stretching vibrations, asymmetric and symmetric, respectively. The major peak at 1745 cm^{-1} was generated from C=O stretching vibrations; the bands at 1457 and 1372 cm^{-1} were incited from CH_2 and CH_3 scissoring vibration, while those bands located at 1235, 1163 cm^{-1} were associated with the C–O stretching vibration. A peak around 914 cm^{-1} was assigned to HC=CH, (*cis*), bending out-of-plane.

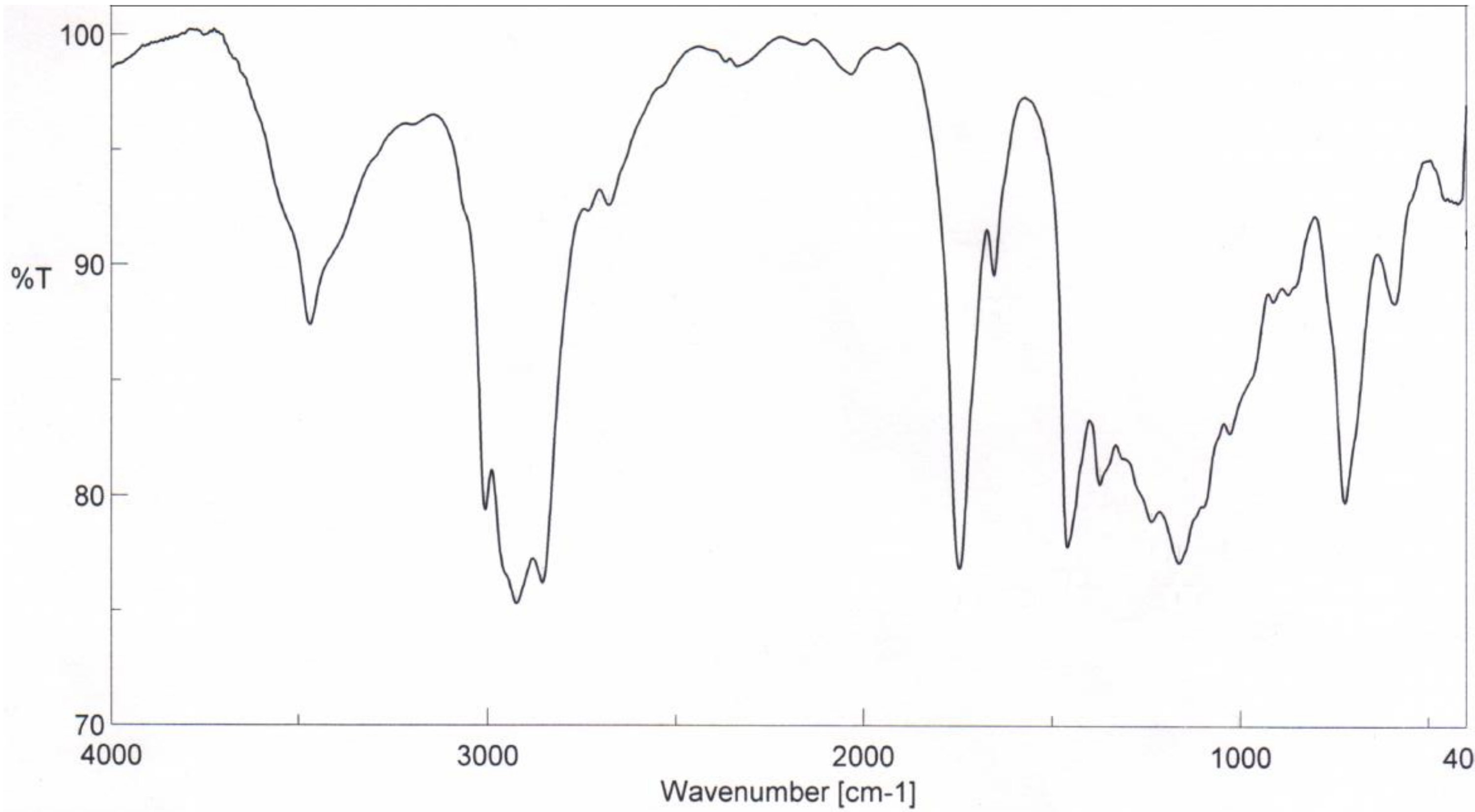


Figure (3) FTIR transmittance spectrum of fresh AKO

Table (17) FTIR spectral data and assignments of the FTIR signals of the main components of fresh AKO

FTIR spectral data of fresh AKO		**Assignments of the FTIR signals**
cm^{-1}	**Intensity %**	
3469.31	87.3935	Overtone of glyceride ester C=O
3005.52	79.3658	=CH (olefinic group of USFA)
2923.56	75.3163	C–H stretching (asymmetry)
2854.13	76.1921	C–H stretching (symmetry)
2678.64	92.5909	C=O Fermi resonance
1743.33	76.8607	C=O stretching
1652.7	89.5673	C=C stretching (*cis*)
1459.85	77.791	C–H bending (scissoring)
1374.03	80.5058	C–H bending
1236.15	78.9139	C–H bending
1163.83	77.1165	C–O stretching and C–H bending
1029.8	82.7388	C–H bending
913.129	88.4632	HC=CH (*cis*) bending out-of-plane
873.596	88.7771	C–C stretching
721.247	79.7332	C–H bending (rocking)

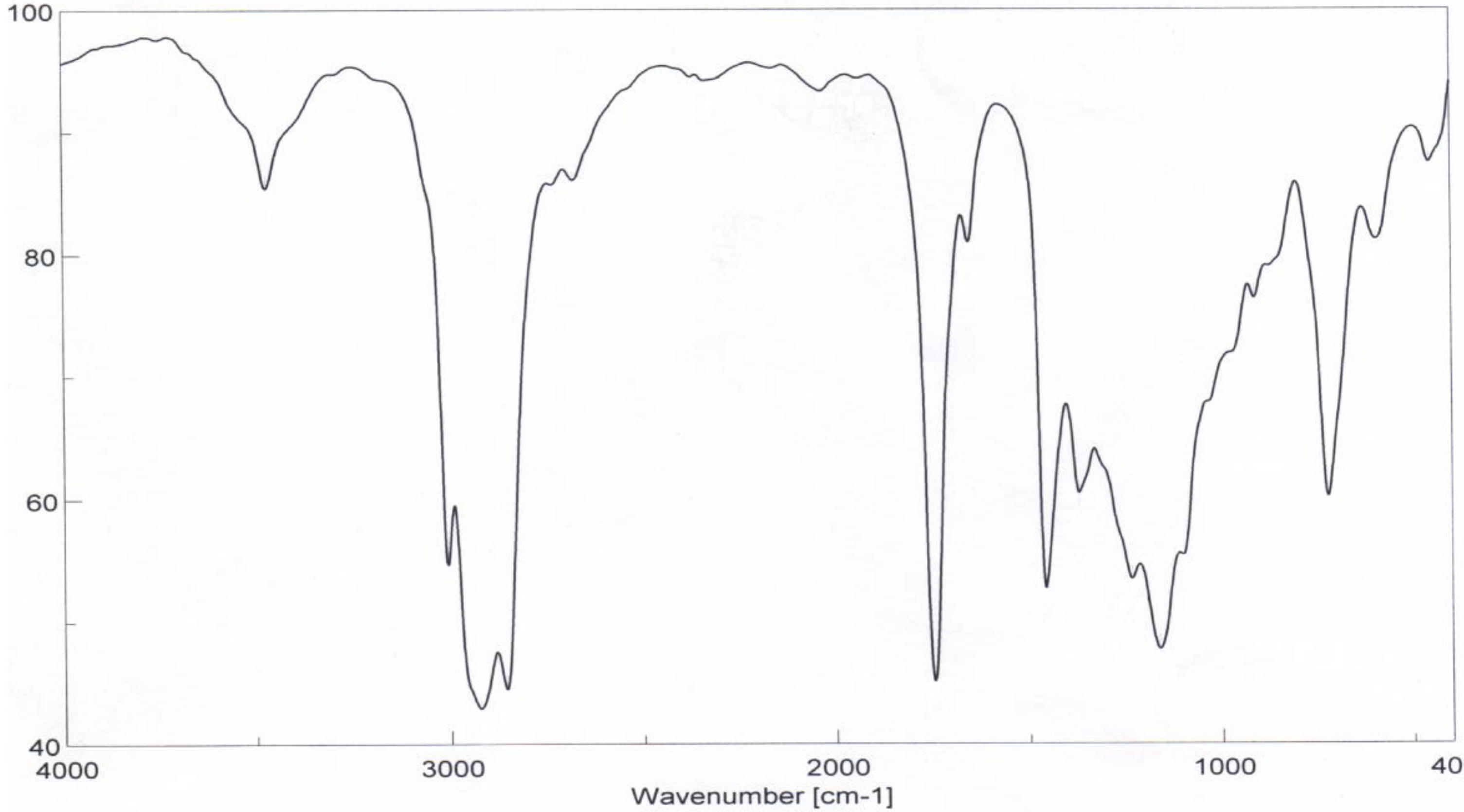


Figure (4) FTIR transmittance spectrum of fresh SO

Table (18) FTIR spectral data and assignments of the FTIR signals of the main components of Fresh SO

FTIR spectral data of fresh SO		**Assignments of the FTIR signals**
cm^{-1}	**Intensity %**	
3470.28	85.4249	Overtone of glyceride ester C=O
3006.48	54.6473	=CH (olefinic group of USFA)
2924.52	42.9071	C–H stretching (asymmetry)
2856.06	44.4795	C–H stretching (symmetry)
2677.68	86.0543	C=O Fermi resonance
1745.26	45.0991	C=O stretching
1653.66	80.8875	C=C stretching (*cis*)
1475.92	52.6722	C–H bending (scissoring)
1372.1	60.4656	C–H bending
1235.18	53.4545	C–H bending
1163.83	47.7482	C–O stretching and C–H bending
914.093	76.3238	HC=CH (*cis*) bending out-of-plane
722.211	60.1971	C–H bending (rocking)

It is well known that every oil or fat differs in composition, length and unsaturated degree of the acyl chains as well as their positions. As shown in **Figures (1-4)** and **Tables (15-18)**, the entire range of spectra looks almost similar for all oils unless one observed very closely. This was due to their similar base chemical composition. However, when considering carefully the FTIR transmittance spectra of the fresh extra virgin olive; moringa; apricot kernel and sunflower oils, one can find valuable differences in their FTIR spectral data concerning the intensities of predominant peaks and the wavenumber shifts due to difference in oil compositions.

In fact, there are some differences in the position and the number of the FTIR peaks of oils under investigation. However, the FTIR peaks of all non-oxidized oils indicated the stretching and pending of aliphatic and unsaturated hydrocarbon in the fatty acids.

The aforementioned major predominant peaks were present in all samples but with varying intensities. Also, there were probably some wavenumber shifts for the same chemical groups in the different studied oil samples which were attributed to many acceptable reasons that will not affect the identity of the chemical groups as described formerly by **Gauglitz and Vo-Dinh (2003).**

If one evaluates each spectrum, there was a slight difference observed in the FTIR spectra of oils under investigation in terms of transmittances at a wavenumber around 3005 cm^{-1} (3003-3006 cm^{-1}), corresponding to the vibration of *cis*-olefinic double bonds in the stretching mode. This observation comes to pass with **Rohman *et al.* (2014).**

From the same **Figures (1-4)** and **Tables (15-18)**, the triglyceride, which is a major component in edible oils and fats, was dominant in the spectra. The main peaks that represent triglyceride functional groups were observed around 2924 cm^{-1} (2923-2929 cm^{-1}) assigned to C–H stretching (asymmetry), around 2854 cm^{-1} (2854-2856 cm^{-1}) due to C–H stretching (symmetry), around 1745 cm^{-1} (1743-1746 cm^{-1}) due to C=O stretching, around 1653 cm^{-1} (1653-1655 cm^{-1}) attributed to the presence of C=C stretching (*cis*), around 1459 cm^{-1} (1457-1460 cm^{-1}) assigned to C–H bending (scissoring), around 1163 cm^{-1} (1162-1163 cm^{-1}) due to C–O stretching and C–H bending, and around 722 cm^{-1} (721-722 cm^{-1}) due to C–H bending (rocking). These findings are in conformity with those of **Yang *et al.* (2005); Vlachos *et al.* (2006).**

It was noticed that the spectrum of the studied oils indicated the presence of *cis*–CH=CH– group (peak at 722 cm^{-1} for extra virgin olive, moringa, sunflower oils and at 721 cm^{-1} for apricot kernel oil) and the absence of 962 cm^{-1} peak indicated the absence of *trans*–CH=CH– group from the fatty acids.

In this respect, **Reder *et al.* (2014)** decided that the spectral band in the range 2990–2885 cm^{-1} is attributed to the stretching vibrations of C–H bond in the aliphatic chain of fatty acids. In spectral region 1770–1725 cm^{-1} the absorption of –C=O stretching vibration occurs. The bands in spectral regions 1479–1453 cm^{-1} and 1426–1409 cm^{-1} are linked with $–CH_2–$ scissoring and –CH= *(cis)* bending. However, **Guillén and Cabo (1997); Muik *et al.* (2007); Rohman *et al.* (2014)** deduced that the region selected between 1800 and 1400 cm^{-1} mostly represents the combination of C–H bending, C=O stretching, and C=C stretching and hence is directly related to unsaturated C=C bond. It is demonstrated that vibrations of C–H bending, C=O stretching, and C=C stretching played a very important role in discriminant analysis.

As seen, most of the spectral information was compressed in the wavenumber regions of 3100–2800 cm^{-1} and 1800–900 cm^{-1}. It was visible that the prominent peaks were due to C–H stretching in the wavenumber region of 3100–2800 cm^{-1}, C=O stretching in the region of 1800–1700 cm^{-1} and C–O–C stretching, C–H bending in the region of 1400–900 cm^{-1}. It was clearly noticed that there were certain variations between the spectra of all oils under investigation in the fingerprint wavenumber region of 1200–800 cm^{-1}. These results showed an agreement compatible with those reported by **Tay *et al.* (2002); Lerma-García *et al.* (2011).**

In particular, as mentioned before, the lipid acyl chains absorbed between 3030 and 2800 cm^{-1} (around 3005, 2925, 2854 cm^{-1}) and between 1500 and 1350 cm^{-1} (around 1460, 1373 cm^{-1}), while around 1745 cm^{-1} the ester carbonyl IR response was observed. A component around 3005 cm^{-1} that was due to the olefinic group (=CH) indicated the presence of unsaturated fatty acids. A component appeared around 1373 cm^{-1}, assigned to CH_3 bending. These obtained results fall in line with those reported by **Ami *et al.* (2014).**

The band developed at 3006 cm^{-1} in the FTIR spectra of SO is a fingerprint of the oxidation process associated with the C–H stretching vibration of *cis*-double bond (=CH). The band position remains almost unaltered or undergoes a very low shifting towards higher wavenumber during the first time of oxidation that involved the increase of hydroperoxides content and of some secondary oxidation compounds. However, a shift towards lower wavenumbers and the decrease of its amplitude may be revealed up to complete quenching at very advanced stages of the oxidation which characterized by the breaking of ester bonds between the fatty acids and glycerol, that decreases the unsaturation degree and increases the *trans* isomers concentration. These effects can be attributed to the disappearance of the *cis*-double bond of the unsaturated acyl groups.

These aforementioned results obtained from FTIR spectra of fresh oils are in accordance with those reported by **Guillén and Cabo (1997); Bertran *et al.* (1999); Moreno *et al.* (1999); Yang and Irudayaraj (2001); Sinelli *et al.* (2007); Gurdeniz and Ozen (2009); Moros *et al.* (2009); Sherazi *et al.* (2009); Al-Degs *et al.* (2011); Navarra *et al.* (2011); Gómez-Caravaca *et al.* (2013).**

It is well known that the region 1800-1630 cm^{-1} is related to the vibration of the carbonyl group of triacylglycerol esters. It is normally observed in the FTIR spectra of fresh non-oxidized edible oils that both aliphatic and carbonyl groups give a very strong absorption in the corresponding regions. The more oxidized samples demonstrated a higher absorption in this zone.

As shown above, one can draw a conclusion that all the fresh oils used in this investigation showed many similarities between their FTIR spectral data concerning wavenumbers or intensities or shapes of peaks which make the differentiation between them very difficult. However, when was closely examined, the FTIR spectral data, some variations concerning some wavenumbers and the intensity of absorption were found if compared at the same specified wavenumber. These variations successfully led to the differentiation between oils under study. There were some individual peaks with characteristic wavenumbers which were identical with their corresponding oils. These characteristic peaks include the shift of absorption peaks around 3005 cm^{-1} assigned to –*cis* CH=CH within the four oils under investigation. It was observed the shift of this peak to different wavenumbers either to higher or to smaller values which could reflect some parameter responsible for the internal structure and consequent effect on stability of oils. The oils under study showed the following shift of absorption peak around 3005 cm^{-1}: extra virgin olive oil at 3004 cm^{-1}, moringa oil at 3003 cm^{-1}, apricot kernel oil at 3005 cm^{-1} and sunflower oil at 3006 cm^{-1}. These results could reflect the same order of oil stability evaluated by the common Rancimat method; where the observed shifts arranged moringa oil (3003 cm^{-1}) at the first order followed by extra virgin olive oil (3004 cm^{-1}), then apricot kernel oil (3005 cm^{-1}) and at least sunflower oil (3006 cm^{-1}). One can briefly state this result as an arrangement of oils stability descendingly as follows: MO > EVOO > AKO > SO.

With regard to **Figure (5)** and **Table (19)** the FTIR spectra of oxidized olive oil had authority over peaks around 3448, 3003, 2924, 2855, 2679, 2368, 2034, 1745, 1648, 1458, 1375, 1236, 1162, 877 and 722 cm^{-1}. In oxidized oils, a band of hydroperoxides was detected at approximately 3444 cm^{-1}, which may be shifted to 3448 cm^{-1}

differentiating the oxidized oil from non-oxidized one. Absorbencies at 2924 and 2855 cm^{-1} were due to bands resulted from CH_2 stretching vibrations, asymmetric and symmetric, respectively. The major peak at 1745 cm^{-1} was work up from C=O stretching vibrations; the band at 1375 cm^{-1} aroused from CH_3 scissoring vibration, while bands located at 1236, 1162 cm^{-1} were associated with the C–O stretching vibration. A small peak was found at 722 cm^{-1} which was corresponded to CH_2 rocking mode.

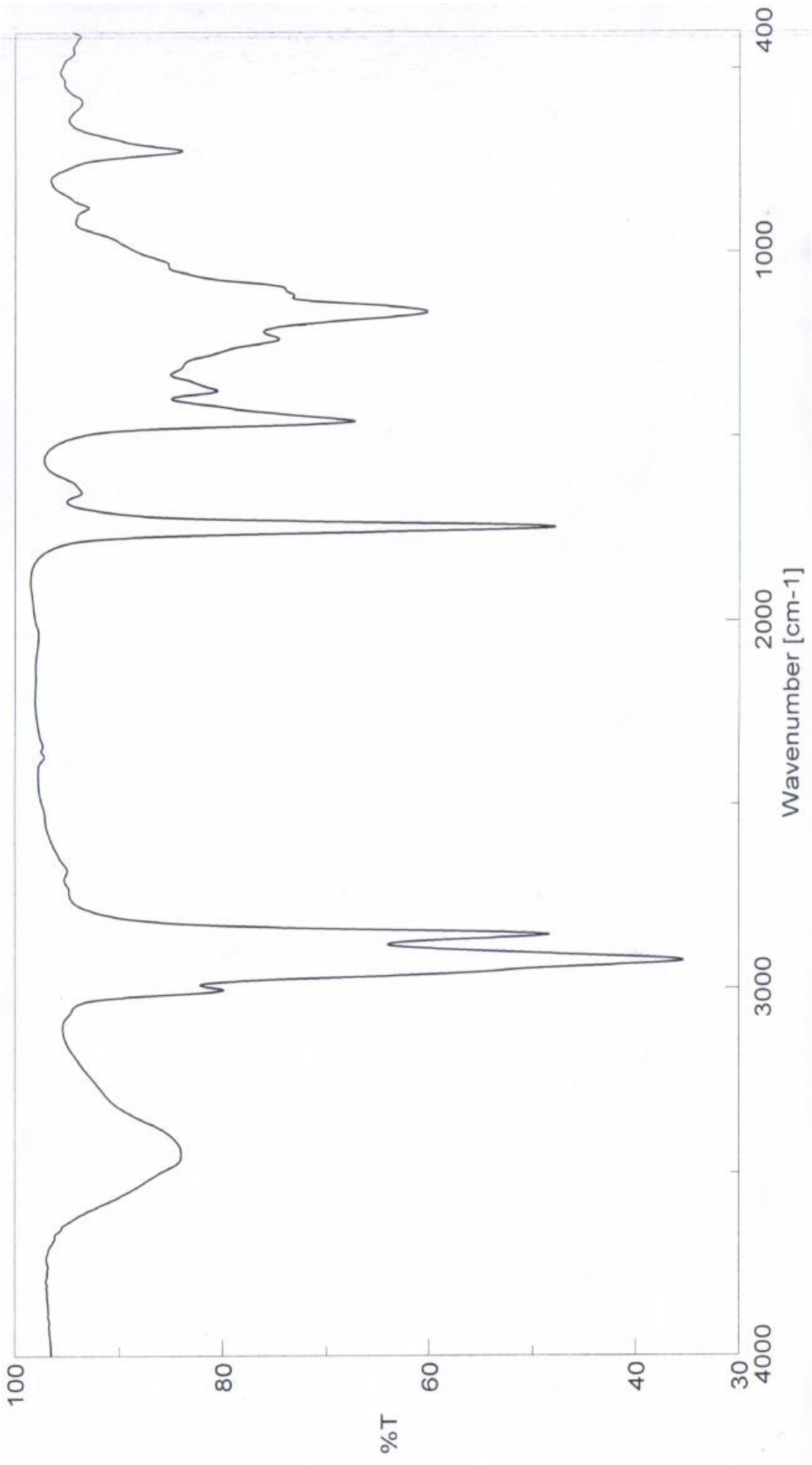


Figure (5) FTIR transmittance spectrum of oxidized olive oil

Table (19) FTIR spectral data and assignments of the FTIR signals of the main components of oxidized olive oil (Ox-OO)

FTIR spectral data of Ox-OO		**Assignments of the FTIR signals**
cm^{-1}	**Intensity %**	
3448.1	83.9867	O-H stretching of hydroperoxide
3003.59	79.8448	=CH (olefinic group of USFA)
2924.52	35.3582	C–H stretching (asymmetry)
2855.1	48.3253	C–H stretching (symmetry)
2679.6	94.9373	C=O Fermi resonance
2368.16	97.1561	O-C=O stretching (asymmetry)
1745.26	47.7584	C=O stretching
1648.84	93.5604	C=C stretching (*cis*)
1458.89	67.136	C–H bending (scissoring)
1375	80.4252	C–H bending
1236.15	74.4869	C–H bending
1162.87	60.097	C–O stretching and C–H bending
877.452	92.8925	C–C stretching
722.211	83.8695	C–H bending (rocking)

Table (20) Comparison of FTIR spectral data of fresh studied oils and oxidized olive oil (Ox-OO)

EVOO		MO		AKO		SO		Ox-OO	
cm^{-1}	**Int. %**	**cm^{-1}**	**Int. %**	**cm^{-1}**	**Int. %**	**cm^{-1}**	**Int. %**	**cm^{-1}**	**Int. %**
3470.28	84.9656	3471.24	83.2993	3469.31	87.3935	3470.28	85.4249		
								3448.1	83.9867
3004.55	41.1342	3003.59	39.4357	3005.52	79.3658	3006.48	54.6473	3003.59	79.8448
2924.52	5.15742	2924.52	16.5768	2923.56	75.3163	2924.52	42.9071	2924.52	35.3582
2854.13	8.08297	2854.13	18.1319	2854.13	76.1921	2856.06	44.4795	2855.1	48.3253
2732.64	85.6821	2733.6	82.9933						
2678.64	85.718	2678.64	82.8497	2678.64	92.5909	2677.68	86.0543	2679.6	94.9373
2378.76	93.5382								
								2368.16	97.1561
				2337.3	98.6258	2334.41	94.1699		
		2319.95	96.7564						
2306.45	93.1692								
		2155.06	98.5777	2156.99	99.5735	2159.88	95.1904		
2030.68	92.1193	2030.68	96.2798	2031.64	98.2892	2031.64	93.2963	2034.53	97.7101
						1936.18	94.3476		
1746.23	8.27813	1745.26	18.3263	1743.33	76.8607	1745.26	45.0991	1745.26	47.7584
1653.66	82.343	1654.62	83.1733	1652.7	89.5673	1653.66	80.8875	1648.84	93.5604
1460.81	27.2903	1460.81	27.473	1459.85	77.791	1475.92	52.6722	1458.89	67.136
1373.07	46.7211	1373.07	41.861	1374.03	80.5058	1372.1	60.4656	1375	80.4252
1236.15	32.5847	1236.15	31.4455	1236.15	78.9139	1235.18	53.4545	1236.15	74.4869
1163.83	16.7683	1162.87	21.9025	1163.83	77.1165	1163.83	47.7482	1162.87	60.097
1118.51	34.5927	1117.55	31.9718						
1098.26	36.1165								
1031.73	62.0355	1030.77	56.1103	1029.8	82.7388				
				913.129	88.4632	914.093	76.3238		
870.703	77.558	871.667	75.2416	873.596	88.7771			877.452	92.8925
722.211	51.1663	722.211	46.0406	721.247	79.7332	722.211	60.1971	722.211	83.8695
						594.932	81.1179		
588.182	82.7427	588.182	83.5534	588.182	88.3837			587.218	93.4946
458.011	86.2429	457.047	86.9853			455.118	87.421		
432.941	86.376								
				422.334	92.6917				
								409.799	93.6298

The infrared spectra of the non-oxidized oils under study **(Tables 15-18)**, showed a band near 3470 cm^{-1} which was associated with the overtone of the glyceride ester carbonyl absorption (–C=O). It is well known that, as the oxidation process advances, the concentration of hydroperoxide groups in the oxidized oil sample increases and also its absorption in the infrared spectrum. This functional group gives a broad band, so it overlaps with that of the overtone of the glyceride ester groups, producing a decreasing in the frequency value of the glyceride original band together with an increasing in its absorbance. For this reason, frequency and absorbance of this band can give information about the hydroperoxide generation throughout the oxidation process. It was noticed some small variations concerning the shift of peak at wavenumber around 3470 cm^{-1} as follows: extra virgin olive oil had its peak at 3470.24 cm^{-1} with intensity of 84.9656, moringa oil had its peak at 3471.24 cm^{-1} of intensity 83.2993, apricot kernel oil at 3469.31 cm^{-1} of intensity 87.3935 and sunflower oil has its peak at 3470.28 cm^{-1} of intensity 85.4249.

On the other hand, it is observed the absence of this peak (3470 cm^{-1}) in the oxidized olive oil sample (**Figure 5** and **Table 19**) which agreed well with the deteriorated status of this oxidized oil, i.e. the oxidized oil will not have any sort of this peak (3470 cm^{-1}).

Regarding the comparison of FTIR spectral data for fresh oils (extra virgin olive; moringa; apricot kernel and sunflower oils) and oxidized olive oil in **Table (20)**, all the spectra of non-oxidized oils under study showed a small band near to 3470 cm^{-1}, as mentioned above. However in oxidized olive oil, only, a band of hydroperoxides was detected at approximately 3448 cm^{-1} differentiating the oxidized oil from non-oxidized one. During oil oxidation reactions there is a considerable growth of the ROOH absorption band, which is observed around 3448 cm^{-1} in the spectra of low-PV oils and shifts progressively to lower wavenumbers as oxidation proceeds. The oxidized olive oil sample exhibited a band located at 3448.1 cm^{-1}, which is attributed to the very weak O-H stretching of the hydroperoxide group. Results obtained are get on with those reported by **Guillén and Cabo (1997); Russin *et al.* (2004); Maggio *et al.* (2009).**

The above observations could not present comparable relations between the shift intensity of peak and the stability of oil when using this parameter as differentiating one (as in the case of 3005 cm^{-1} mentioned above), because it was noticed the similarity in values of wavenumber of the peak of extra virgin olive oil (at 3470.28 cm^{-1} of intensity 84.9656) which has a considerable high stability, with that of sunflower oil (peak observed at 3470.28 cm^{-1} of intensity 85.4249) which is the last oil in the order of stability and considered as low-stability edible oil. So we can not use this parameter (shift of peak at 3470 cm^{-1}) to distinguish between edible oils of different stabilities. But one can draw a conclusion that this peak is present only in non-oxidized oils and could be a useful preliminary indicator of stability, i.e. when the oil show this peak around 3470 cm^{-1}, consequently it is an indication that the oil is non-oxidized one.

In FTIR spectrum, the band of *cis*- double bonds appeared near 3005 cm^{-1} (assigned to =C–H groups) in non-oxidized oil samples, its frequency was related to the composition of the oil; oils with a large proportion of polyunsaturated acyl groups have higher frequency values (such as 3006.48 cm^{-1}) than those with a large proportion of monounsaturated or saturated acyl groups. During the oxidation process, the disappearance of *cis*- double bonds is produced, as well as isomerization of *cis*- to *trans*- groups, alongside with hydroperoxide generation. For this reason, frequency and absorbance of band near 3005 cm^{-1} suffer changes as the oxidation process advances. In this respect, **Russin *et al.* (2003)** observed that the time at which the frequency of this band begins to decrease is also a measurement of the oil stability.

There were other bands, near 1236, 1163 and 1118 cm^{-1}, related to the stretching vibrations of the C–O groups of esters, whose frequency values showed an evolution similar to that observed in band near 3005 cm^{-1}, throughout the oxidation process.

In Connection to the shift and intensity of peaks around 1236 cm^{-1} (assigned for $–CH_2–$), moringa oil of the highest stability as evaluated by Rancimat method, had a peak at 1236.15 cm^{-1} of intensity 31.4455 compared with extra virgin olive oil which arranged in the second order of stability after moringa oil, having a similar peak at 1236.15 cm^{-1} of intensity 32.5847, apricot kernel oil had also a peak at 1236.15 cm^{-1} of intensity 78.9139, being in the third order, however, sunflower oil had a peak at 1235.18 cm^{-1} of intensity 53.4545, holding a position in the fourth order, then oxidized olive oil having a peak at 1236.15 cm^{-1} of intensity 74.4869. It was noticed that moringa, extra virgin olive, apricot kernel and oxidized olive oils produced the peak of 1236.15 cm^{-1} at the same wavenumber but with variable intensities which were ascendingly arranged as follows: moringa oil (31.4455), extra virgin olive oil (32.5847), apricot kernel oil (78.9139) then oxidized olive oil (74.4869). These results could reflect the same order of oils stability as evaluated by Rancimat method (MO > EVOO > AKO > Ox-OO). Only sunflower oil was out of this order, which showed an absorption peak at 1235.18 cm^{-1} of intensity 53.4545. This shift to lower wavenumber than other studied oils along with higher intensity of absorption peak agreed with the order of its lowest stability evaluated by the common Rancimat method. These findings are keeping with those reported by **Guillén and Cabo (2002).**

The FTIR spectral data analysis of the oxidized olive oil showed that this band around 1236 cm^{-1} related to $–CH_2$ and C–O groups, which was more affected by oxidation. When comparing the intensity of peaks around this wavenumber, the oxidized olive oil showed the similar peak at 1236.15 cm^{-1} of intensity 74.4869 which was much higher than the doublet of the intensity (32.5847) of the same peak around the same wavenumber of the non-oxidized fresh extra virgin olive oil. These results go well with those reported by **Maggio *et al.* (2011).**

Regarding the shift and intensity of peaks around 1163 cm^{-1} (assigned for –C–O–), moringa oil of the highest stability as evaluated by the common Rancimat method, had a peak at 1162.87 cm^{-1} of intensity 21.9025 compared with extra virgin olive oil which came in the second order of stability after moringa oil, having a peak at 1163.83 cm^{-1} of intensity 16.7683. Both apricot kernel and sunflower oils produced the same wavenumber at 1163.83 cm^{-1} with intensities of 77.1165 and 47.7482, being in the third and fourth orders, then oxidized olive oil having a peak at 1162.87 of intensity 60.097. It was noticed that extra virgin olive oil had a peak at higher wavenumber shift at 1163.83 cm^{-1} but with lower intensity of 16.7683, compared with moringa oil which showed a peak at lower wavenumber shift at 1162.87 cm^{-1} along with higher intensity of 21.9025 compared with extra virgin olive oil. These findings may reflect the order of extra virgin olive oil as following moringa oil in stability. While, apricot kernel and sunflower oils showed the same shift of peak at 1163.83 cm^{-1} just as extra virgin olive oil with much higher intensities.

So, it is concluded that moringa oil which was evaluated as an oil of the highest stability by the common Rancimat method, showed the lowest shift of absorption peak at 1162.87 cm^{-1} with comparatively low intensity than sunflower oil which was evaluated as oil of lowest stability by the common Rancimat method. However, oxidized olive oil caused the same value of absorption peak as moringa oil (1162.87 cm^{-1}), but it had much higher intensity of this peak approaching 3 times as moringa oil which could differentiate it from moringa oil. These findings may reflect the relation between oil of high stability and the lower shift and lower intensity of absorption peak at 1163 cm^{-1} as reported by **Guillén and Cabo (2002).**

Concerning the shift and intensity of peaks around 1118 cm^{-1} as shown in **Tables (15-20)**, moringa oil of the highest stability as evaluated by the common Rancimat method, had a peak at 1117.55 cm^{-1} of intensity 31.9718 compared to extra virgin olive oil which came in the second order in stability having a peak at 1118.51 cm^{-1} of intensity 34.5927. These peaks were given only in the FTIR spectra of moringa and extra virgin olive oils, where it was noticed that the extra virgin olive oil induced a peak at higher wavenumber shift of 1118.51 cm^{-1} along with higher intensity of 34.5927, compared to moringa oil which showed a peak at lower wavenumber shift of 1117.55 cm^{-1} along with lower intensity of 31.9718. This finding may reflect the order of extra virgin olive oil as following moringa oil in their stability. These results agreed with those reported by **Guillén and Cabo (2002).**

For the evaluation of oxidative stability of edible oils by FTIR spectroscopy, we should consider the region 1800-1630 cm^{-1} which is related to the vibration of the carbonyl group of triacylglycerol esters. As shown in **Table (20)**, the non-oxidized oil samples showed the band of the triglyceride ester groups near 1745 cm^{-1}. It was deducted by **Guillén and Cabo (2002)** that during the oxidation process hydroperoxides degrade into secondary oxidation products such as aldehydes and ketones, which give bands near 1728 cm^{-1}. These bands overlap with that of ester group at 1745 cm^{-1} causing a broadening of the band and a decreasing of its frequency. In this study, it was observed the shift of the absorption peak at 1745 cm^{-1} (assigned to –C=O) to lower wavenumbers among moringa, extra virgin olive, apricot kernel and sunflower oils of different stabilities at 1745.26 cm^{-1} of intensity 18.3263, 1746.23 cm^{-1} of intensity 8.27813, 1743.33 cm^{-1} of intensity 76.8807, and at 1745.26 cm^{-1} of intensity 45.0991, respectively. However, the oxidized olive oil produced a peak at 1745.26 cm^{-1} of intensity 47.7584. It was deduced that this relationship may be useful when comparing an oil of low stability as sunflower oil which showed lower wavenumber (1745.26 cm^{-1}) than extra virgin olive oil (1746.23 cm^{-1}) which had higher stability compared to sunflower oil, but this was not the same regular case to be taken as a rule because it was found that extra virgin olive oil gave higher shift (1746.23 cm^{-1}) than moringa oil (1745.26 cm^{-1}) which is considered to be the highest-stability among oils in this study. Also, apricot kernel oil

(1743.33 cm^{-1}) had higher stability than sunflower oil but it caused lower shift of peak than sunflower oil. These observations were applicable when comparing sunflower oil of low stability with extra virgin olive oil of high stability, but it was not applicable when comparing sunflower oil with moringa and apricot kernel oils which were also of high stability. So these results related to the shift and intensity of absorption peak around 1745 cm^{-1} might be correlated to other parameters in the composition of the edible oils under investigation.

It is normally observed in the infrared spectrum of fresh extra virgin olive oil that both aliphatic and carbonyl groups give a very strong absorption in the corresponding regions. To differentiate between oxidative stability of the fresh extra virgin olive oil and the oxidized olive oil, as shown from the comparison of their FTIR spectral data in **Table (20)**, the more oxidized samples demonstrated a high absorption in this zone. A strong band at 1746.23 cm^{-1} with intensity of 8.27813 (assigned to –C=O) and a small peak at 1653.66 cm^{-1} of intensity of 82.343 (assigned to *cis* CH=CH) for fresh extra virgin olive oil were observed. On the other hand, the oxidized olive oil showed a strong band at 1745.26 cm^{-1} with intensity of 47.7584 (assigned to –C=O) and a small peak at 1648.84 cm^{-1} with intensity of 93.5604 (assigned to *cis* CH=CH). It was noticed the shift to smaller wavenumbers in case of oxidized sample, i.e. from 1746.23 cm^{-1} for extra virgin olive oil to 1745.26 cm^{-1} for oxidized olive oil and from 1653.66 cm^{-1} for EVOO to 1648.84 cm^{-1} for oxidized olive oil. These obtained results showed an agreement with those reported by **Gómez-Caravaca *et al.* (2013).**

As mentioned by **Maggio *et al.* (2009)** and **Navarra *et al.* (2011)**, there are two interesting spectral regions: (i) from 700 to 1500 cm^{-1}, where the vibrational activity of conjugated bonds and of bending vibrations of aliphatic compounds are observed and (ii) from 2800 to 3800 cm^{-1}, where the activity of stretching vibrations of fatty acids and hydroperoxides is observed.

Of particular interest, was the appearance of a component around 3005 cm^{-1} due to the olefinic group (=CH). This indicated the presence of unsaturated fatty acids as mentioned by **Ami *et al.* (2014).** If we examined the FTIR spectra of all oils under investigation (**Tables 15-20**), concerning the absorption peak and its shift and intensity around 3005 cm^{-1}, we can deduce a useful relationship between this intensity and the unsaturated fatty acids content of oils. From the fatty acid analysis, the highest value of unsaturated fatty acids was recorded for apricot kernel oil (94.39722) followed by sunflower oil (87.20838), extra virgin olive oil (82.56549) then moringa oil (76.85655). On the other hand, the intensity of absorption peak in FTIR spectral data around 3005 cm^{-1} showed the same order, where, moringa oil had a peak at 3003.59 cm^{-1} of intensity 39.4357, extra virgin olive oil had a peak at 3004.55 cm^{-1} of intensity 41.1342, sunflower oil gave a peak at 3006.48 cm-1 of intensity 56.6473 and apricot kernel oil had a peak at 3005.52 cm^{-1} of intensity 79.3658. The intensities of peaks around 3005 cm^{-1} of the studied oils versus their unsaturated fatty acids content are summarized in **Table (21).**

From this table, it was clearly noticed a positive relationship between the peak intensities around 3005 cm^{-1} and the olefinic groups (=CH) presented in oils; it means that the higher in unsaturated fatty acids, the higher of this peak intensity. From this relation, it was easy to predict the order and the approximate values of the unsaturated fatty acids of edible oils under study from the intensity of peak around 3005 cm^{-1}.

In the FTIR spectral data, if we examined the shift of absorption peaks around 3005 cm^{-1} for oils under study, we found the values of theses shifts of absorption peaks could be also correlated to the polyunsaturated fatty acids content for each corresponding oil. These observations are shown in **Table (22)**, which lists the values of polyunsaturated fatty acids for each oil along with its shift of absorption peak around 3005 cm^{-1}.

Table (21) Unsaturated fatty acids % and intensity of absorption peak around 3005 cm^{-1} of the studied oils

Oil	Unsaturated fatty acids %	Intensity of peak (~ 3005 cm^{-1})
MO	76.86	39.4357
EVOO	82.57	41.1342
AKO	94.40	79.3658
SO	87.21	56.6473

Table (22) Polyunsaturated fatty acids % and shift of absorption peak around 3005 cm^{-1} of the studied oils

Oil	Polyunsaturated fatty acids %	Shift of peak (~ 3005 cm^{-1})
MO	0.96	3003.59
EVOO	11.84	3004.55
AKO	23.56	3005.52
SO	52.55	3006.48

From **Table (22)**, it is obvious that sunflower oil with the highest value of polyunsaturated fatty acids as 52.552% had the highest shift of absorption peak which was at 3006.48 cm^{-1}. Also, sunflower oil showed the lowest oil in its stability according to Rancimat method. In the second order, comes apricot kernel oil which its content of polyunsaturated fatty acids was 23.557% and showed a shift peak at 3005.52 cm^{-1}. Extra virgin olive oil contained 11.84% polyunsaturated fatty acids and gave a shift peak at 3004.55 cm^{-1}. Last, moringa oil which offered the highest stability among the studied oils had only 0.96% of polyunsaturated fatty acids and showed a least shift peak at 3003.59 cm^{-1}. These observations were deducible to correlate the values of polyunsaturated fatty acids percentage of edible oils under investigations with the order of their stability. Also, the shift of absorption peak of the oil at 3005 cm^{-1} could place it in the correct order within a series of oils of different composition. There may be some sort of direct relationship between the polyunsaturated fatty acids value from one hand, and the shift of absorption peaks around 3005 cm^{-1} (assigned to *–cis* CH=CH) from the other hand. But this direct relationship between the polyunsaturated fatty acids value and the shift of absorption peak around 3005 cm^{-1}, will be considered as inversely proportional to the stability of the oil. The oil of highest value of polyunsaturated fatty acids will have highest shift of absorption peak around 3005 cm^{-1} and will show the least stability. On the contrary, the oil with lowest value of polyunsaturated fatty acids will have lowest shift of absorption peak around 3005 cm^{-1} and will show the highest stability.

Table (23) summarizes the relations between polyunsaturated fatty acids (PUFA), the shift of absorption peak around 3005 cm^{-1} and order of stability by the Rancimat methods of edible oils.

Table (23) Polyunsaturated fatty acids % and shift of absorption peak around 3005 cm^{-1} and order of stability by Rancimat methods of the studied oils.

Oil	Polyunsaturated fatty acids %	Shift of peak (~ 3005 cm^{-1})	Order of stability by Rancimat method
MO	0.96	3003.59	1st (Highest stability)
EVOO	11.84	3004.55	2nd
AKO	23.56	3005.52	3rd
SO	52.55	3006.48	4th (Least stability)

From the above results, one draw a conclusion that the order of the stability of edible oils under investigation obtained by FTIR spectral analyses follows the same order evaluated by the common Rancimat methods. The Rancimat method ordered descendingly the four oils under investigation according to their stability as: MO > EVOO > AKO > SO. In the same way, the polyunsaturated fatty acids values arranged these oils where the least value was assigned to the highest stable oil as: MO (0.9604) < EVOO (11.84) < AKO (23.557) < SO (52.552). Concerning the shift of absorption peak around 3005 cm^{-1}, one can also ordered the oils where the lowest shift of the peak was related to the highest oil stability as follows: MO (3003.59) < EVOO (3004.55) < AKO (3005.52) < SO (3006.48).

4.3- Evaluation of Oils Stability by Nuclear Magnetic Resonance (NMR) Spectroscopy

NMR is a physical phenomenon utilized to investigate molecular properties of matter by irradiating atomic nuclei in a magnetic field with radio waves. When exposed to magnetic fields, magnetic nuclei can receive and emit radio waves. The NMR frequency is determined by the magnetic field at the site of the nucleus. Atomic nuclei are surrounded by electrons. The electrons of the chemical bond are shared by different nuclei. Electrons of atoms and molecules move in orbitals. The orbitals of the binding electrons are characteristic of the chemical structure of the molecule. Electrons carry an electric charge. Electric charges in motion induce a magnetic field. The distribution of resonance frequencies forms the NMR spectrum. The NMR spectrum is a fingerprint of the molecular structure, its spectra of molecules in solution is a standard method of analysis.

This type of spectroscopy, mainly proton (^1H) and ^{13}C-NMR, was used for structure elucidation of the organic compounds. Estimations of identification trueness have been made by matching experimental ^1H and ^{13}C spectra of test structures with theoretical spectra predicted for a set of known structures; structures corresponding to the best matching were considered as true results.

The application of NMR to structure analysis is based primarily on the empirical correlation of structure with observed chemical shifts and coupling constants. The magnitude of the chemical shift is expressed in parts per million (ppm) and denoted as delta symbol; δ; it is measured with respect to a reference. For ^1H and ^{13}C spectra the reference material is usually tetramethylsilane, $(CH_3)_4Si$, abbreviated TMS. A very small amount is added internally when organic solvents are used. The position of TMS is assigned as exactly 0.0 on the delta (δ) scale. The chemical shifts are obtained by dividing the frequency difference between the sample and the TMS signal, in hertz, by the TMS frequency in megahertz.

A unique advantage of NMR is that spectra can often be interpreted by using reference data from structurally related compounds. Internal simulation programs allow the NMR spectrum of an unknown to be compared against a postulated structure.

In this study, the elucidation of the chemical structure of the organic compounds of the studied oils was taken as indices to evaluate their stability.

4.3.1- ^{1}H-NMR Spectroscopy

On the basis of nuclear properties alone, all protons having the same chemical surroundings produce signals at the same frequency precess in a fixed magnetic field and would be indistinguishable from each other. Fortunately, in different chemical environments, shifts in resonant frequency arise from partial shielding of the nuclei from the applied magnetic field by the electron cloud around it. The density of this cloud varies with the number and nature of the neighboring atoms. The specific location of the shifted resonant frequencies indicates the chemical nature of the nuclei and provides some information concerning their neighbors. The resonance position of proton spectra is always stated with respect to the resonance of the protons in a reference compound.

4.3.1.1- Evaluation of Oils Stability using ^{1}H-NMR Spectral Data Analysis

The ^{1}H-NMR spectra of the fresh oils (extra virgin olive, moringa, apricot kernel and sunflower oils) are shown in **Figures (6-9). Tables (24-28)** show the chemical shift assignments of the ^{1}H-NMR spectral data of the main components of the studied oils.

Figure (6) and **Table (24)** show that the ^{1}H-NMR spectrum of fresh extra virgin olive oil aroused peaks at 0.780 ppm assigned to $-CH_3$ (C18-steroid group) and attributed to β-sitosterol; 0.858 ppm assigned to $-CH_3$ (acyl group) and attributed to saturated, oleic and linoleic acids; two peaks at 1.172, 1.242 ppm assigned to methylene protons

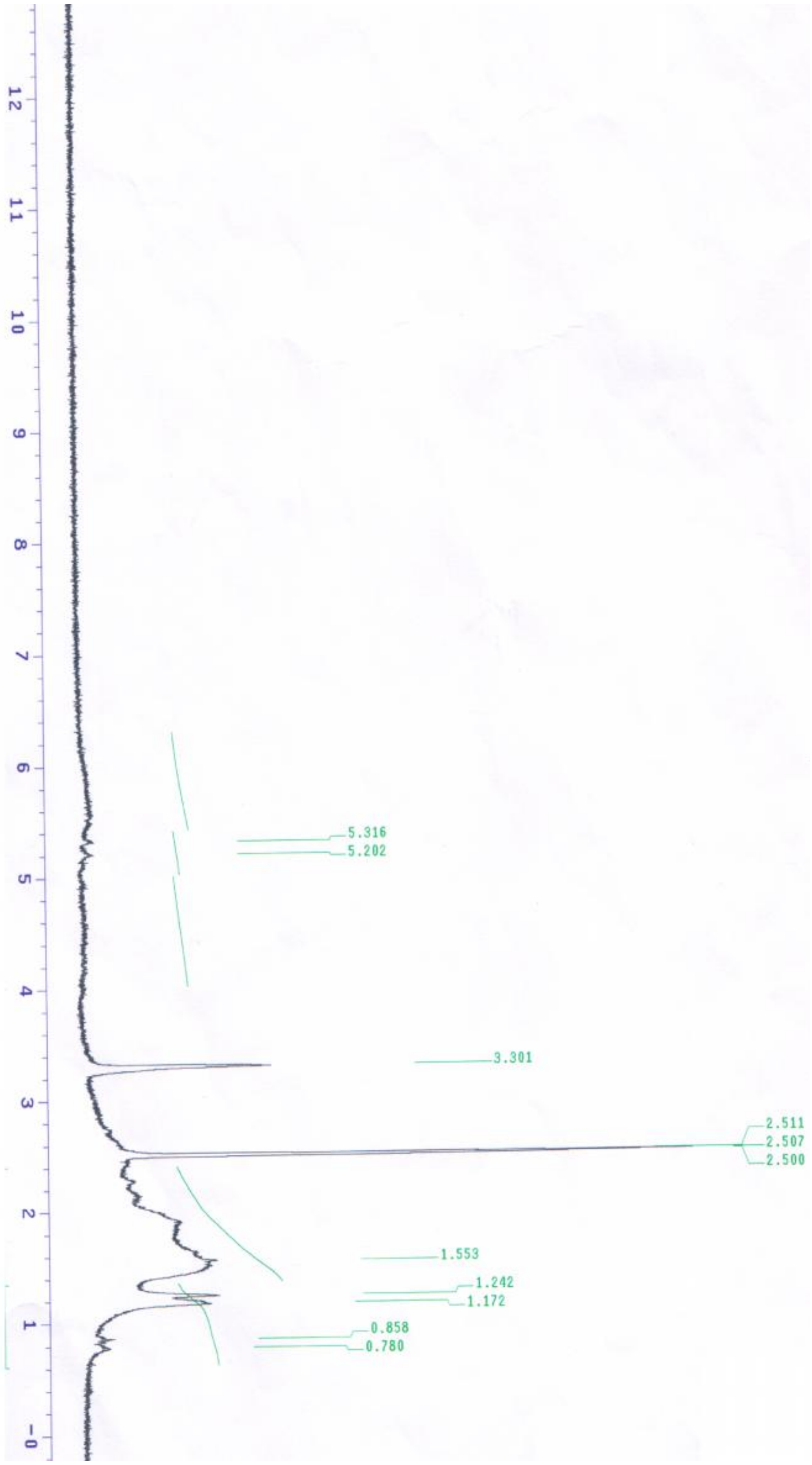


Figure (6) ^{1}H-NMR spectrum of fresh EVOO in DMSO-d6

Table (24) Chemical shift (δ) assignment of ^{1}H-NMR signals of the main components of fresh EVOO in DMSO-d6

δ (ppm)	**Functional group**	**Assignment**
0.780	$-CH_3$ (C18-steroid group)	β-sitosterol
0.858	$-CH_3$ (acyl group)	sat., oleic and linoleic
1.172	$-(CH_2)_n-$ (acyl group)	aliphatic chains
1.242	$-(CH_2)_n-$ (acyl group)	aliphatic chains
1.553	$-OCO-CH_2-CH_2-$ (acyl group)	oleyl acyl chains
2.500	DMSO-d6	solvent DMSO-d6
2.507	DMSO-d6	solvent DMSO-d6
2.511	DMSO-d6	solvent DMSO-d6
3.301	DMSO-d6	H_2O of DMSO –d6
5.202	>CHOCOR (glyceryl group)	triglycerides
5.316	$-CH=CH-$ (acyl group)	unsaturated fatty acids

of $-(CH_2)_n-$ (acyl group) and attributed to aliphatic chains; a major peak at 1.553 ppm with a considerable intensity of 58.18% characterizes extra virgin olive oil assigned to $-OCO-CH_2-CH_2-$ (acyl group) and attributed to oleyl acyl chains; three peaks at 2.500, 2.507, 2.511 ppm assigned to deuterated dimethyl sulphoxide (DMSO-d6) and attributed to DMSO solvent; a peak at 3.301 ppm assigned to DMSO-d6 and attributed to the water of DMSO-d6 solvent; a peak at 5.202 ppm assigned to >CHOCOR (glyceryl group) and attributed to triglycerides and a peak at 5.316 ppm and assigned to $-CH=CH-$ (acyl group) which attributed to unsaturated fatty acids.

Extra virgin olive oil showed very small concentrations of linoleic acyl group, but it had a predominant concentration of oleic (intensity of the signal at 1.553 ppm). It was also evident that extra virgin olive oil did not contain linolenic (Ln) acyl groups (absence of signals at 0.972, 2.093, 2.111, and 2.801 ppm), but it contained an infrequent concentration of linoleic (L) acyl groups (intensity of the signal at 0.858 ppm).

It is deduced from **Figure (7)** and **Table (25)** that the ^{1}H-NMR spectrum of fresh moringa oil developed peaks around 0.662 ppm assigned to $-CH_3$ (C18-steroid group) and attributed to stigmasterol; 0.784 ppm assigned to $-CH_3$ (C18-steroid group) and attributed to β-sitosterol; 0.843, 0.866, 0.942, 964 ppm assigned to $-CH_3$ (acyl group) and attributed to saturated, oleic and linoleic acids; 1.073, 1.185, 1.248, 1.406, 1.481 ppm assigned to $-(CH_2)_n-$ (acyl group) and attributed to aliphatic chains; 1.794 ppm assigned to $>C=C–CH_3$ (allylic group) and attributed to allyl chain; 1.925, 2.017, 2.144 ppm assigned to $–CH_2–CH=CH–$ (acyl group) and attributed to unsaturated fatty acids; 2.262, 2.306 ppm assigned to $–OCO–CH_2–$ (acyl group) and attributed to oleyl acyl chains; 2.505 ppm assigned to DMSO-d6 and attributed to DMSO-d6 solvent; 3.278 ppm assigned to DMSO-d6 and attributed to the water of DMSO-d6 solvent; 3.875 ppm assigned to $–CH_2OH$ (glyceryl group) and attributed to *sn*-1,2 diacylglycerol; 4.022 ppm assigned to >CH–OH and attributed to *sn*-1,3 diacylglycerol; 5.094 ppm assigned to $–CH_2–O–CO–R$ and attributed to *sn*-1,3 diacylglycerol; 5.138, 5.215 ppm assigned to >CHOCOR (glyceryl group) and attributed to triglycerides; 5.327 ppm and assigned to −CH=CH− (acyl group) and attributed to unsaturated fatty acids and 7.370 ppm assigned to –CH=CH– (olefinic protons) and attributed to olefins.

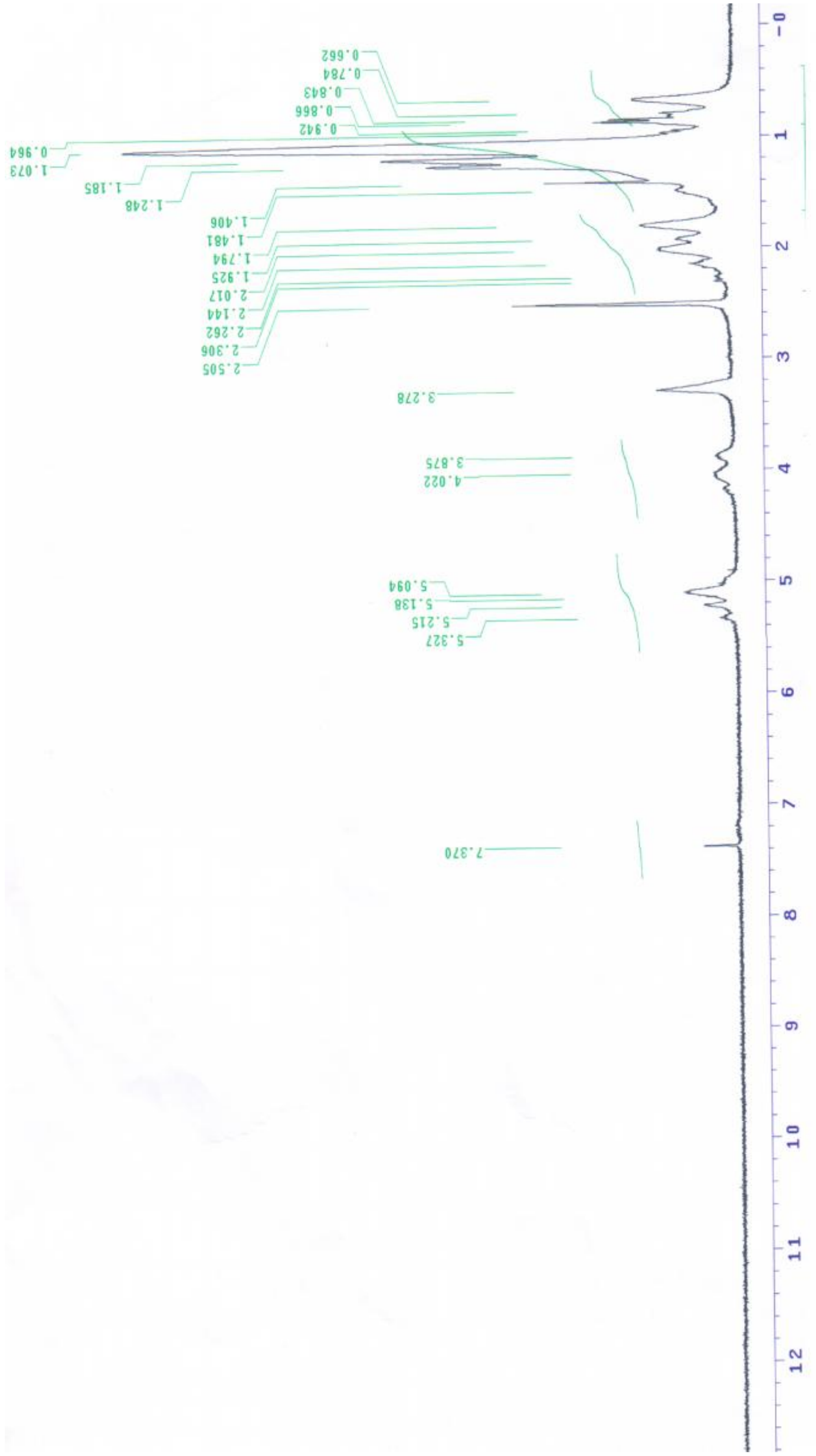


Figure (7) ^{1}H-NMR spectrum of fresh MO in DMSO-d6

Table (25) Chemical shift (δ) assignment of ^{1}H-NMR signals of the main components of fresh MO in DMSO-d6

δ (ppm)	Functional group	Assignment
0.662	$-CH_3$ (C18-steroid group)	stigmasterol
0.784	$-CH_3$ (C18-steroid group)	β-sitosterol
0.843	$-CH_3$ (acyl group)	sat., oleic and linoleic
0.866	$-CH_3$ (acyl group)	sat., oleic and linoleic
0.942	$-CH_3$ (acyl group)	sat., oleic and linoleic
0.964	$-CH_3$ (acyl group)	sat., oleic and linoleic
1.073	$-(CH_2)_n-$ (acyl group)	aliphatic chains
1.185	$-(CH_2)_n-$ (acyl group)	aliphatic chains
1.248	$-(CH_2)_n-$ (acyl group)	aliphatic chains
1.406	$-(CH_2)_n-$ (acyl group)	aliphatic chains
1.481	$-(CH_2)_n-$ (acyl group)	aliphatic chains
1.794	$>C=C–CH_3$ (allylic group)	allyl chain
1.925	$–CH_2–CH=CH–$ (acyl group)	unsaturated fatty acids
2.017	$–CH_2–CH=CH–$ (acyl group)	unsaturated fatty acids
2.144	$–CH_2–CH=CH–$ (acyl group)	unsaturated fatty acids
2.262	$–OCO–CH_2–$ (acyl group)	oleyl acyl chains
2.306	$–OCO–CH_2–$ (acyl group)	oleyl acyl chains
2.505	DMSO-d6	solvent DMSO-d6
3.278	DMSO-d6	H_2O of DMSO-d6
3.875	$–CH_2OH$ (glyceryl group)	*sn*-1,2 diacylglycerol
4.022	$>CH–OH$	*sn*-1,3 diacylglycerol
5.094	$–CH_2–O–CO–R$	*sn*-1,3 diacylglycerol
5.138	>CHOCOR (glyceryl group)	triglycerides
5.215	>CHOCOR (glyceryl group)	triglycerides
5.327	–CH=CH– (acyl group)	unsaturated fatty acids
7.370	–CH=CH– (olefinic protons)	olefins

Moringa oil had very small concentrations of linolenic and linoleic acyl groups and a considerable amount of oleic (intensity of signals at 0.843, 0.866, 0.942, 0.964, 2.262 and 2.306 ppm). It was clear that moringa oil did not contain linolenic (Ln) acyl groups (absence of signals at 0.972, 2.093, 2.111, and 2.801 ppm).

As shown in **Figure (8)** and **Table (26)**, the ^{1}H-NMR spectrum of fresh apricot kernel oil gave rise to peaks around 0.809, 0.819, 0.840, 0.861, 0.880, 0.939 ppm assigned to $-CH_3$ (acyl group) and attributed to saturated, oleic and linoleic acids; 0.960 ppm assigned to $-CH_3$ (acyl group) and attributed to linolenic acid; 1.252, 1.401, 1.478 ppm assigned to $-(CH_2)_n-$ (acyl group) and attributed to aliphatic chains; 2.008, 2.179 ppm assigned to $–CH_2–CH=CH–$ (acyl group) and attributed to unsaturated fatty acids; 2.303, 2.497 ppm assigned to $–OCO–CH_2–$ (acyl group) and attributed to oleyl acyl chains; 2.503, 2.508 ppm assigned to DMSO-d6 and attributed to DMSO-d6 solvent; 2.739 assigned to $=CH–CH_2–CH=$ (acyl group) and attributed to polyunsaturated fatty acids; 3.309 ppm assigned to DMSO-d6 and attributed to the water of DMSO-d6 solvent; 4.245, 4.901 ppm assigned to $–CH_2OCOR$ (glyceryl group) and attributed to *sn*-1,3 diacylglycerol; 5.327 ppm assigned to –CH=CH– (acyl group) and attributed to unsaturated fatty acids and 7.368 ppm assigned to –CH=CH– (olefinic protons) and attributed to olefins.

Apricot kernel oil had small concentrations of linolenic and linoleic acyl groups, but it had an extremely concentration of oleic (intensity of signals at 0.809, 0.819, 0.840, 0.861, 0.880, 0.939, 2.303 and 2.497 ppm). It was also evident that AKO oil had not linolenic (Ln) acyl groups (absence of signals at 0.972, 2.093, 2.111, and 2.801 ppm). However, it aroused a peak at 0.960 ppm (shifted from 0.972 ppm) assigned to $–CH_3$ (acyl group) which was attributed to the appearance of linolenic acid with very small intensity indicating a trace of this acid. But AKO showed a quite concentration of linoleic (L) acyl groups (intensity of the signal at 0.858 ppm).

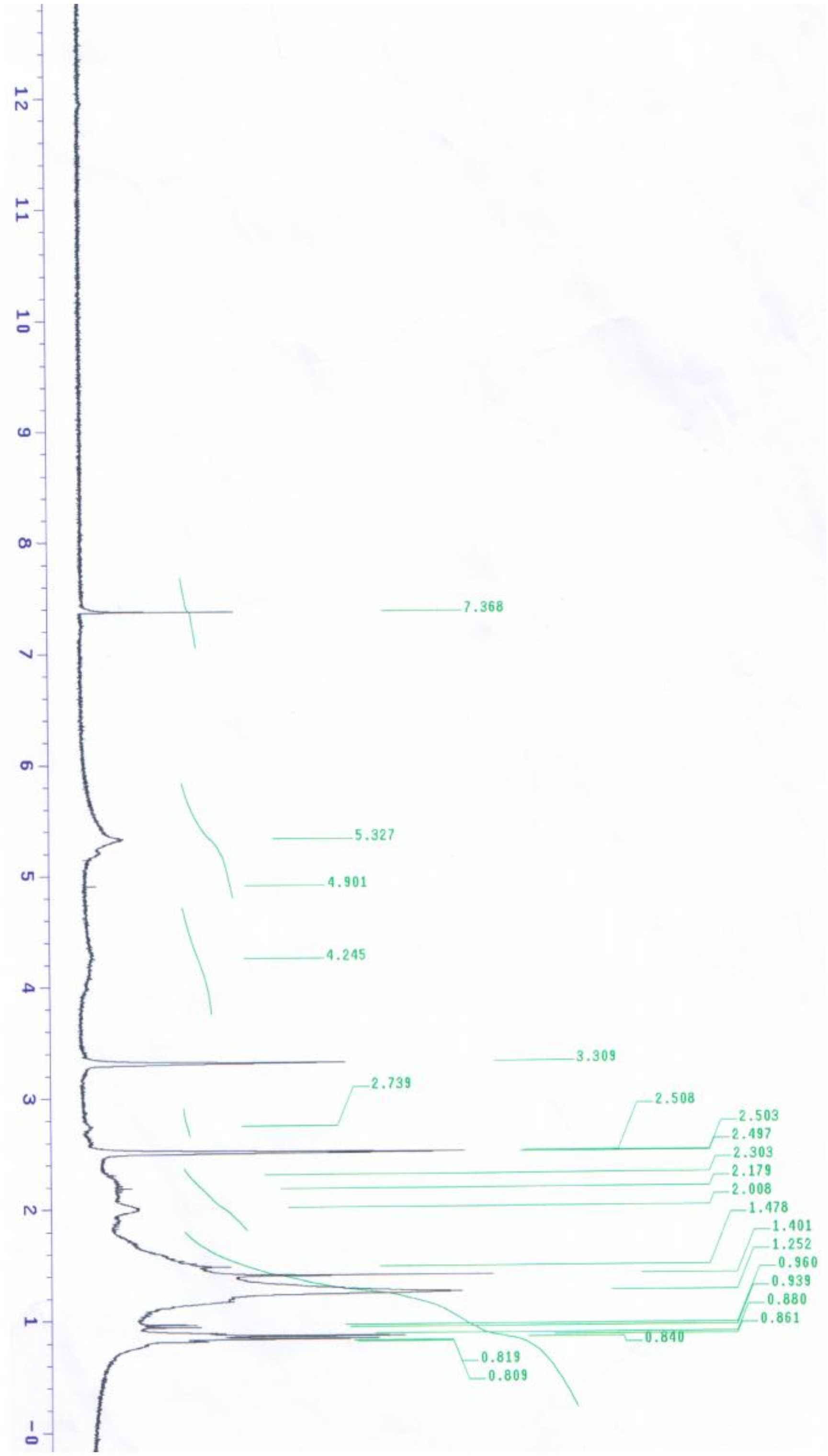


Figure (8) ^{1}H-NMR spectrum of fresh AKO in DMSO-d6

Table (26) Chemical shift (δ) assignment of ^{1}H-NMR signals of the main components of fresh AKO in DMSO-d6

δ (ppm)	**Functional group**	**Assignment**
0.809	$-CH_3$ (acyl group)	sat., oleic and linoleic
0.819	$-CH_3$ (acyl group)	sat., oleic and linoleic
0.840	$-CH_3$ (acyl group)	sat., oleic and linoleic
0.861	$-CH_3$ (acyl group)	sat., oleic and linoleic
0.880	$-CH_3$ (acyl group)	sat., oleic and linoleic
0.939	$-CH_3$ (acyl group)	sat., oleic and linoleic
0.960	$-CH_3$ (acyl group)	linolenic
1.252	$-(CH_2)_n-$ (acyl group)	aliphatic chains
1.401	$-(CH_2)_n-$ (acyl group)	aliphatic chains
1.478	$-(CH_2)_n-$ (acyl group)	aliphatic chains
2.008	$–CH_2–CH=CH–$ (acyl group)	unsaturated fatty acids
2.179	$–CH_2–CH=CH–$ (acyl group)	unsaturated fatty acids
2.303	$–OCO–CH_2–$ (acyl group)	oleyl acyl chains
2.497	DMSO-d6	solvent DMSO-d6
2.503	DMSO-d6	solvent DMSO-d6
2.508	DMSO-d6	solvent DMSO-d6
2.739	$=CH–CH_2–CH=$ (acyl group)	polyunsaturated fatty acids
3.309	DMSO-d6	H_2O of DMSO-d6
4.245	$–CH_2OCOR$ (glyceryl group)	*sn*-1,3 diacylglycerol
4.901	$–CH_2OCOR$ (glyceryl group)	*sn*-1,3 diacylglycerol
5.327	$–CH=CH–$ (acyl group)	unsaturated fatty acids
7.368	$–CH=CH–$ (olefinic protons)	olefins

From **Figure (9)** and **Table (27)** it was clearly noticed that the ^{1}H-NMR spectrum of fresh sunflower oil originated peaks around 0.767, 0.854 ppm assigned to $-CH_3$ (acyl group) and attributed to saturated, oleic and linoleic acids; 1.160, 1.437 ppm assigned to $-(CH_2)_n-$ (acyl group) and attributed to aliphatic chains; 1.896, 2.106 ppm assigned to $–CH_2–CH=CH–$ (acyl group) and attributed to unsaturated fatty acids; 2.492, 2.497 ppm assigned to $–OCO–CH_2–$ (acyl group) and attributed to oleyl acyl chains; 2.503, 2.510, 2.516 ppm assigned to DMSO-d6 and attributed to DMSO-d6 solvent; 2.627 ppm assigned to $-CH_3$ (acyl group) and attributed to linoleic acid; 3.317 ppm assigned to DMSO-d6 and attributed to the water of DMSO-d6 solvent; 3.968 ppm assigned to $–CH_2OH$ (glyceryl group) and attributed to *sn*-1,2 diacylglycerol; 4.149 ppm assigned to $–CH_2OCOR$ (glyceryl group) and attributed to *sn*-1,3 diacylglycerol and 5.184 ppm assigned to $-CH=CH-$ (acyl group) and attributed to unsaturated fatty acids.

Sunflower oil had an inconsiderable concentration of linolenic acyl group, while it had exceedingly oleic and linoleic (intensity of signals at 0.767, 0.854, 2.492 and 2.497 ppm). SO did not contain linolenic (Ln) acyl groups (absence of signals at 0.972, 2.093, 2.111, and 2.801 ppm), but it had an abundant concentration of linoleic (L) acyl groups (intensity of a signal at 2.627 ppm).

Sunflower oil could be easily distinguished from the other edible oils under investigation due to its high linoleic acid content which was particularly evident in the intensity of the signal resonating at 2.627 ppm. Protons of linoleic acid gave visible signals at 0.854 ppm, and at 2.06 ppm, due, respectively, to the terminal methyl group and to the allylic protons of linoleic acid ($C_{18:2}$).

Also, linolenic acid ($C_{18:3}$) contains two inner allylic methylene groups ($–CH=CH–CH_2–CH=CH–CH_2–CH=CH–$). Thus double bonds in $C_{l8:3}$ are twice and likely to shift and form a conjugated diene than linoleic acid ($C_{18:2}$).

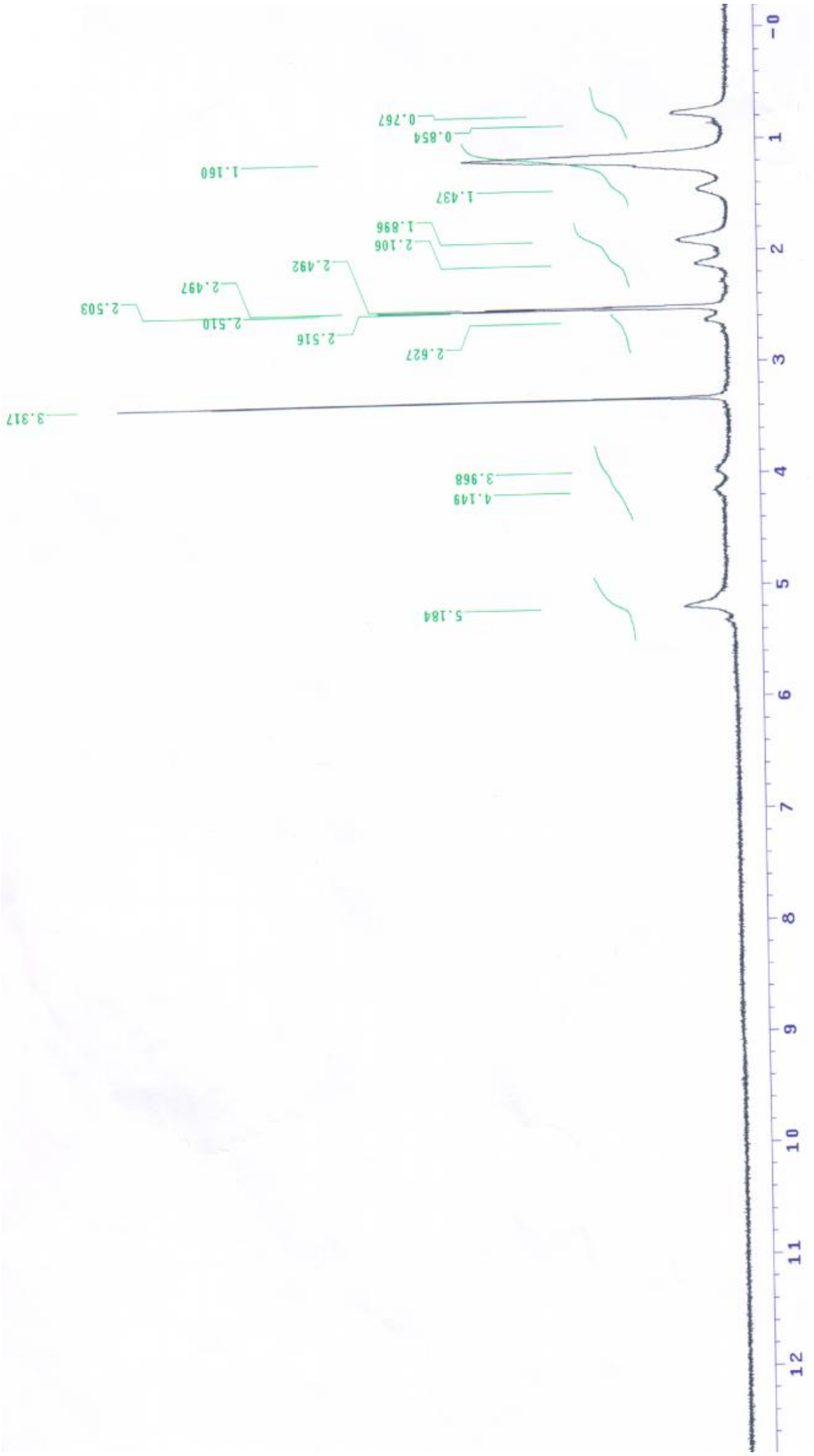


Figure (9) ^{1}H-NMR spectrum of fresh SO in DMSO-d6

Table (27) Chemical shift (δ) assignment of ^{1}H-NMR signals of the main components of fresh SO in DMSO-d6

δ (ppm)	**Functional group**	**Assignment**
0.767	$-CH_3$ (acyl group)	sat., oleic and linoleic
0.854	$-CH_3$ (acyl group)	sat., oleic and linoleic
1.160	$-(CH_2)_n-$ (acyl group)	aliphatic chains
1.437	$-(CH_2)_n-$ (acyl group)	aliphatic chains
1.896	$–CH_2–CH=CH–$ (acyl group)	unsaturated fatty acids
2.106	$–CH_2–CH=CH–$ (acyl group)	unsaturated fatty acids
2.492	$–OCO–CH_2–$ (acyl group)	oleyl acyl chains
2.497	$–OCO–CH_2–$ (acyl group)	oleyl acyl chains
2.503	DMSO-d6	solvent DMSO-d6
2.510	DMSO-d6	solvent DMSO-d6
2.516	DMSO-d6	solvent DMSO-d6
2.627	$-CH_3$ (acyl group)	linoleic
3.317	DMSO-d6	H_2O of DMSO-d6
3.968	$–CH_2OH$ (glyceryl group)	*sn*-1,2 diacylglycerol
4.149	$–CH_2OCOR$ (glyceryl group)	*sn*-1,3 diacylglycerol
5.184	$–CH=CH–$ (acyl group)	unsaturated fatty acids

From the results obtained, it could be noticed that the chemical shifts of oil signals were positive with respect to TMS and were characteristic of each particular molecular environment. Edible oils are mainly made up of triglycerides, with different substitution patterns due to length, degree and kind of unsaturation of the acyl groups, and minor components such as *mono*- and *di*-glycerides, sterols, fatty acids, and others. If we consider the high field NMR spectra, various resonances can be assigned to specific chemical groups. In particular, the peaks around 4.2 ppm aroused from ^{1}H nuclei attached to carbon at positions 1 and 3 on the glycerol backbone. This is a useful group of peaks, as it provides a measure of the overall glyceride concentration in the sample, and can be employed as a reference signal. The set of peaks around 5.2 ppm (5.2-5.6 ppm) arises largely from the ^{1}H nuclei attached to carbons involved in a double bond, usually referred to as –CH=CH– (olefinic protons). This signal is related to the total number of unsaturated bonds in a triglyceride, regardless of whether these are located within mono-unsaturated or polyunsaturated chains. The very small signals around 2.7 ppm (2.6-2.9 ppm) arise from bis-allylic protons from the $–CH_2–$ group located between pairs of unsaturated bonds and thus provide a measure of the number of poly-unsaturated fatty acid chains present in the sample.

The deuterated dimethyl sulphoxide (DMSO-d6) solvent component of each sample gave rise to two prominent resonances at a chemical shifts around 2.5 and 3.3 ppm. The first one of these two resonances was assigned to the solvent DMSO-d6 peak, whereas, the second resonance was assigned to the water of DMSO-d6 solvent.

The band around 1.3 ppm in the NMR spectra arises from $–CH_2$ groups more than two chemical bonds away from a functional group, such as –C=C– (or the ester coupling group linking the fatty acid chain to the triglyceride backbone). The number of such ‘conventional’ $–CH_2$ groups will vary with the number of double bonds in the fatty acid chains. Considering the band in the range 1.8-2.1 ppm, the relevant NMR spectral feature for this band is $–CH_2–CH=CH$ (the allylic protons), and is likely to be associated with a relatively high levels of monounsaturated oleic acid. These results agreed with those reported by **Guillén and Ruiz, (2003a, 2003b); Vigli *et al.* (2003); Alonso-Salces *et al.* (2011); Parker *et al.* (2014)**.

Concerning oils minor components in this study, in addition to main components, non-oxidized oils also contain minor components, which are also important because some of them can show antioxidant stability. Some of these compounds were present in high enough concentrations to be detected by ^{1}H-NMR, and the signals of their protons did not overlap with those of the main lipid components. Thus, this technique provided information about them. Some of these detected components in the studied oils are shown in **Tables (24, 25).** Extra virgin olive oil contained β-sitosterol, whereas, moringa oil contained stigmasterol and β-sitosterol.

From the **Figures (6-9)**, it was noticed the apparent differences between the ^{1}H-NMR spectra of the different studied edible oils (EVOO, MO, AKO, SO). These differences help in the identification of the oxidative stability of each edible oil as its ^{1}H-NMR fingerprint. It was also noticed that the ^{1}H-NMR signals of hydroperoxides (primary oxidation products) and aldehydes (main secondary oxidation products) were not detected in the oils under this study indicating that no oxidative degradation was taking place. The presence of these oxidation products in the ranges of 8.09-8.19 ppm for hydroperoxides proton, and 9.30-9.90 ppm for aldehydes, indicates oxidation of these edible oils according to **Alonso-Salces et al. (2011)**.

The most interesting ^{1}H signals are likely to be those of the methyl groups. The methyl groups of the linolenic acyl chains (methyl groups of n-3 fatty acids) appeared slightly different to the methyl groups of the other fatty acid chains. Because these vegetable oils contain less linolenic acid than others, it is possible to use this information to discriminate among these oils.

Also, the ^{1}H-NMR spectra of the oils showed the signals corresponding to the major components in these oils. It is well known that every oil differs in composition, length and unsaturated degree of the fatty acids as well as their positions in the chain. The triglyceride, which is a major component in edible oils and fats, was dominant in the spectra. Because oil stability varies from one oil to another and depends on its triacylglycerol composition as well as the presence of different minor components in the oils, correlation between the obtained results and the stability of the corresponding oils was carried out.

The comparison of ^{1}H-NMR spectral data of the fresh edible oils under investigation **(Table 28)** showed that the glycerol and unsaturated protons for all oils were appeared between 4.10 and 5.40 ppm, whereas, saturated protons signals appeared between 0.80 and 2.80 ppm. Proton signals of the methylene group which directly adjacent to the carbonyl group of fatty acids (α-carbonyl-CH_2) resonating at 2.2–2.4 ppm were found only in moringa and apricot kernel oils. For moringa oil, the allylic and olefinic protons, owing to oleic, linoleic and linolenic groups, in relation to that of methylene protons in the α-position nearby the carbonyl group was observed at 1.794 ppm for allylic protons $>C=C–CH_3$, and at 7.370 ppm for olefinic protons. Moringa and apricot kernel oils also had apparent proportions of olefinic protons resonating at 7.3 ppm. It was noticed that apricot kernel oil had higher proportion of olefinic protons than moringa oil. It had apparent linoleic acid content which was particularly evident in the intensity of the signal resonating at 2.739 ppm.

Table (28) Comparison of chemical shift (δ) assignment of ^{1}H-NMR signals of the main components of the fresh studied oils in DMSO-d6

δ (ppm)	Assignment of ^{1}H-NMR signals of the main components of oils			
	EVOO	MO	AKO	SO
0.662		stigmasterol		
0.767				sat., $C_{18:1}$, $C_{18:2}$
0.780	β-sitosterol			
0.784		β-sitosterol		
0.809			sat., $C_{18:1}$, $C_{18:2}$	
0.819			sat., $C_{18:1}$, $C_{18:2}$	
0.840			sat., $C_{18:1}$, $C_{18:2}$	
0.843		sat., $C_{18:1}$, $C_{18:2}$		
0.854				sat., $C_{18:1}$, $C_{18:2}$
0.858	sat., $C_{18:1}$, $C_{18:2}$			
0.861			sat., $C_{18:1}$, $C_{18:2}$	
0.866		sat., $C_{18:1}$, $C_{18:2}$		
0.880			sat., $C_{18:1}$, $C_{18:2}$	
0.939			sat., $C_{18:1}$, $C_{18:2}$	
0.942		sat., $C_{18:1}$, $C_{18:2}$		
0.960			$C_{18:3}$	
0.964		sat., $C_{18:1}$, $C_{18:2}$		
1.073		aliphatic chains		
1.160				aliphatic chains
1.172	aliphatic chains			
1.185		aliphatic chains		
1.242	aliphatic chains			
1.248		aliphatic chains		
1.252			aliphatic chains	
1.401			aliphatic chains	
1.406		aliphatic chains		
1.437				aliphatic chains
1.478			aliphatic chains	
1.481		aliphatic chains		
1.553	OL acyl chains			
1.794		allyl chains		
1.896				USFA
1.925		USFA		
2.008			USFA	
2.017		USFA		
2.106				USFA
2.144		USFA		

Table (28) (continued)

δ (ppm)	**Assignments of ^{1}H-NMR signals of the main components of oils**			
	EVOO	**MO**	**AKO**	**SO**
2.179			USFA	
2.262		OL acyl chains		
2.303			OL acyl chains	
2.306		OL acyl chains		
2.492				OL acyl chains
2.497			DMSO-d6	OL acyl chains
2.500	DMSO-d6			
2.503			DMSO-d6	DMSO-d6
2.505		DMSO-d6		
2.507	DMSO-d6			
2.508			DMSO-d6	
2.510				DMSO-d6
2.511	DMSO-d6			
2.516				DMSO-d6
2.627				$C_{18:2}$
2.739			PUFA	
3.278		H_2O of DMSO-d6		
3.301	H_2O of DMSO-d6			
3.309			H_2O of DMSO-d6	
3.317				H_2O of DMSO-d6
3.875		*sn*-1,2 DAG		
3.968				*sn*-1,2 DAG
4.022		*sn*-1,3 DAG		
4.149				*sn*-1,3 DAG
4.245			*sn*-1,3 DAG	
4.901			*sn*-1,3 DAG	
5.094		*sn*-1,3 DAG		
5.138		TG		
5.184				USFA
5.202	TG			
5.215		TG		
5.316	USFA			
5.327		USFA	USFA	
7.368			olefins	
7.370		olefins		

The data in **Table (28)** showed that the DMSO-d6 solvent peaks as 2 apparent peaks slightly different in their chemical shifts around 2.5 ppm and ranging between 2.500-2.497 ppm for the first one and around 3.3 ppm ranging between 3.317-3.278 ppm for the second. The first one of these two resonances was assigned to the solvent DMSO-d6 peak, whereas, the second resonance was assigned to the water of DMSO-d6. As following these peaks, they were triplet at 2.511, 2.507, 2.500 ppm and singlet at 3.301 ppm for extra virgin olive oil; singlet at 2.505 ppm and singlet at 3.278 ppm for moringa oil; triplet at 2.508, 2.503, 2.497 ppm and singlet at 3.309 ppm for apricot kernel oil; and sextet at 2.627, 2.516, 2.510, 2.503, 2.497, 2.492 ppm and singlet at 3.317 ppm for sunflower oil.

It was noticed that the less chemical shift was corresponded to moringa oil followed by extra virgin olive oil, apricot kernel oil then sunflower oil in the last. This order may reflect the stability order of these edible oils and fall in line with the results obtained from the determination of oxidative stability by Rancimat method where the order of oils stability was moringa oil > extra virgin olive oil > apricot kernel oil > sunflower oil. This arrangement also comes in an excellent agreement with the order of tocopherols concentration as powerful natural antioxidant.

The higher oxidative stability of moringa and extra virgin olive oils than other studied oils was attributed to their content of sterols. Moringa oil showed the presence of both stigmasterol and β-sitosterol from the signals at 0.662 and 0.784 ppm, respectively due to methylic protons in position C18 of the steroidal skeleton. Sterols appeared near the more intense signal of the methyl protons of the fatty acids [$-CH_3$ (C18-steroid group)] as shown clearly in the right-hand side inset of **Figure (7).** However, extra virgin olive oil showed a peak at 0.780 ppm assigned to β-sitosterol which is an important marker in olive oil analysis. These signals were attributed to the methyl protons of all sterols at position C18. It has been suggested that a donation of hydrogen atom from the allylic methyl group in the side chain, followed by the isomerization to a relatively tertiary allylic free radical responded the mode of action of the sterol antioxidants. The long induction period of moringa oil might be explained by the presence of sterols. Although, extra virgin olive oil had a higher content of these sterols, it had a shorter induction period. The higher oxidative stability of moringa oil over extra virgin olive oil could be attributed to their fatty acids composition, both extra virgin olive and moringa oils had high oleic acid content, however, extra virgin olive oil contained linoleic and linolenic acids, which were more readily undergoes oxidation and degradation than oleic acid. These acids were in much lower quantities in moringa oil. The higher stability of moringa oil than extra virgin olive oil was also due to other constituents of the non glyceride fraction of the oil which possess antioxidant properties. In addition, moringa oil was the most rich in aliphatic chains. Olefins might be responsible for higher stability of moringa oil since olefins bear an incidental relationship to antioxidants. These results agreed with **Tsaknis *et al.* (1999); Hatzakis *et al.* (2010); Roman *et al.* (2013); Singh (2013); Tranchida *et al.* (2013); Yang *et al.* (2013).**

It could be observed that the high stability of moringa and extra virgin olive oils is mainly due to their relatively low degree of fatty acid unsaturation and to the antioxidant activity of some of the unsaponifiable components such as sterols: β-sitosterol and stigmasterol shown in the oils. These obtained results agreed with **Alonso-Salces *et al.* (2011).**

4.3.1.2- Evaluation of Oils Stability after 6 Months of Storage using ^{1}H-NMR Spectral Data Analysis

Figures (10-13) show the ^{1}H-NMR spectra of the four studied oils (extra virgin olive, moringa, apricot kernel and sunflower oils) after 6 months of storage at room temperature. **Tables (29-32)** show the ^{1}H-NMR spectral data of these stored oils.

From **Figure (10)** and the data in **Table (29)**, it could be noticed that there was some differences between the ^{1}H-NMR spectral data of extra virgin olive oil shown in **Figure (6) and Table (24)**, and this spectral data of extra virgin olive oil after 6 months of storage at room temperature. Considering the stored oil, the same peaks were already shown compared with the fresh oil, except there were shifts to higher or lower values of some chemical shifts (δ). β-sitosterol was appeared in the ^{1}H-NMR spectra of stored oil at 0.776 ppm compared with that of the fresh oil which it appeared at 0.780 ppm. Also, there were some shifts which were for the peak at 0.858 ppm assigned to $–CH_3$ (acyl group) of saturated, oleic and linoleic acids in the fresh oil to 0.853 ppm in the stored oil; for the two peaks at 1.172 and 1.242 ppm assigned to $–(CH_2)_n–$ (acyl group) of the aliphatic chains in EVOO before storage to 1.173 and 1.238 ppm in the stored oil; for the peak at 5.202 ppm assigned to >CHOCOR (glyceryl group) of triglycerides in the fresh oil to 5.204 ppm in the stored oil; and for the peak at 5.316 ppm assigned to −CH=CH− (acyl group) of the unsaturated fatty acids in the fresh oil to 5.318 ppm after storage.

These variations might be attributed to the very slow and slight change in the stability which caused some shifts to lower values of some chemical assignments of ^{1}H-NMR signals. However, the peak at 1.553 ppm assigned to $−OCO−CH_2−CH_2−$ (acyl group) of the oleyl acyl chains which appeared in the fresh extra virgin olive oil was also found in the ^{1}H-NMR spectra of the stored oil at the same peak value of chemical shift and unmoved to any higher or lower value. This peak was only found in the ^{1}H-NMR spectra of olive oil samples and is considered as characteristic peak for this oil.

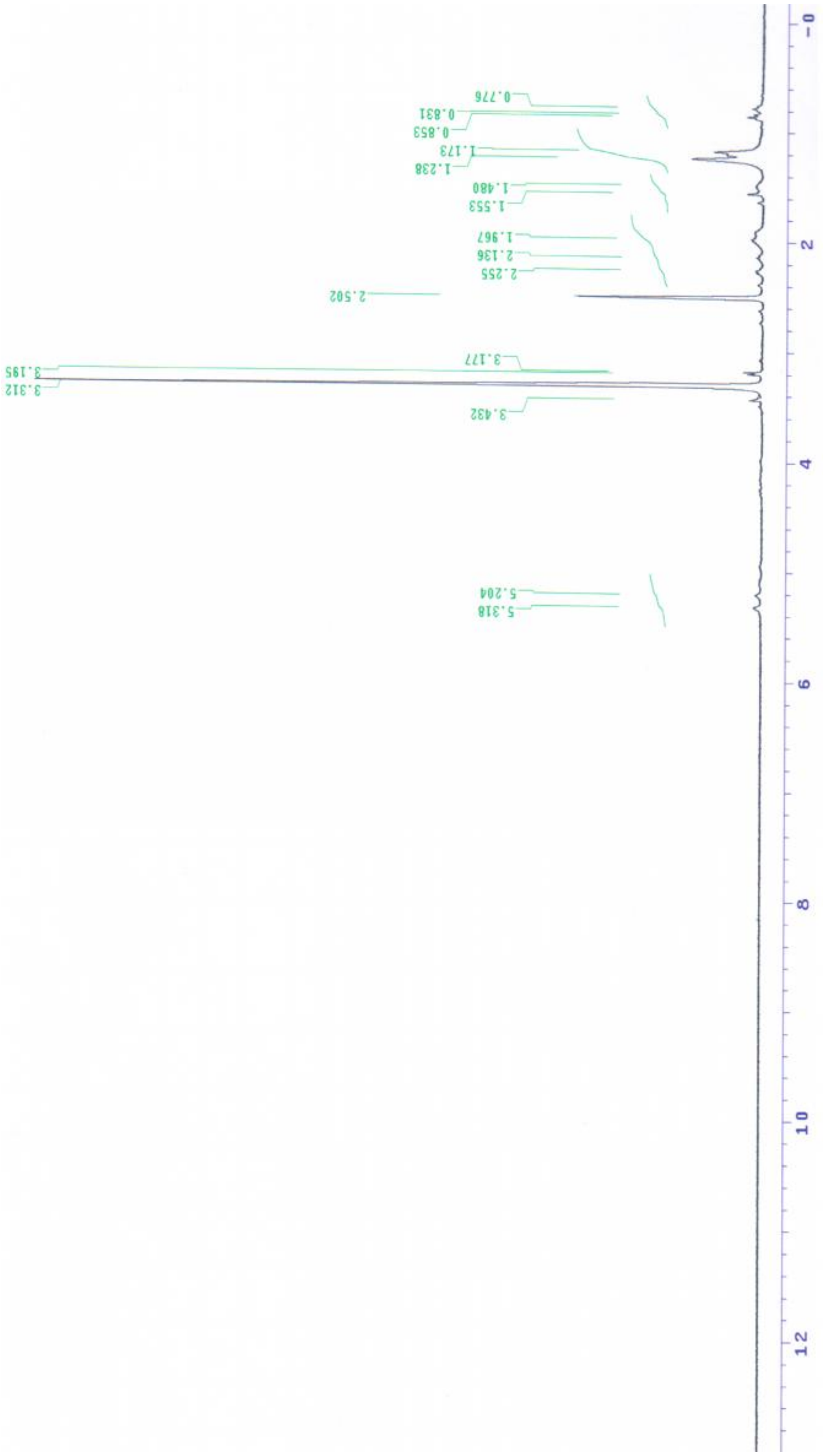


Figure (10) ^{1}H-NMR spectrum of EVOO after storage for 6 months

Table (29) Chemical shift (δ) assignment of ^{1}H-NMR signals of the main components of EVOO after storage for 6 months at room temperature in comparison to the chemical shift of the fresh oil

δ (ppm)		Functional group	Assignment
Fresh oil	**After 6 months**		
0.780	0.776	$-CH_3$ (C18-steroid group)	β-sitosterol
0.858	0.831	$-CH_3$ (acyl group)	sat., $C_{18:1}$ and $C_{18:2}$
	0.853	$-CH_3$ (acyl group)	sat., $C_{18:1}$ and $C_{18:2}$
1.172	1.173	$-(CH_2)_n-$ (acyl group)	aliphatic chains
1.242	1.238	$-(CH_2)_n-$ (acyl group)	aliphatic chains
	1.480	$-(CH_2)_n-$ (acyl group)	aliphatic chains
1.553	1.553	$-OCO-CH_2-CH_2-$ (acyl group)	oleyl acyl chains
	1.967	$-CH_2-CH=CH-$ (acyl group)	unsaturated fatty acids
	2.136	$-CH_2-CH=CH-$ (acyl group)	unsaturated fatty acids
	2.255	$-OCO-CH_2-$	free fatty acids
2.500		DMSO-d6	solvent DMSO-d6
	2.502	DMSO-d6	solvent DMSO-d6
2.507		DMSO-d6	solvent DMSO-d6
2.511		DMSO-d6	solvent DMSO-d6
3.301		DMSO-d6	H_2O of DMSO-d6
	3.312	DMSO-d6	H_2O of DMSO-d6
5.202	5.204	>CHOCOR (glyceryl group)	triglycerides
5.316	5.318	$-CH=CH-$ (acyl group)	unsaturated fatty acids

Also, the solvent peak of DMSO-d6 was aroused as one peak at 2.502 ppm in the stored oil, compared with the fresh one where it was divided into three peaks appeared at 2.500, 2.507, 2.511 ppm (**Figure 6 and Table 24**). This may be attributed to the fact that the fresh oil was more viscous and concentrated than the stored oil, and so the solvent DMSO tried to dissolve the oil to a great extent and permeated the oil structure more deeper than in the case of the stored oil and this made the numerous solvent peak which interfered much more with the fresh oil than it acts with the stored one which was easier to dissolve in the solvent giving rise to one sharp peak at 2.502 ppm. In addition, the solvent DMSO-d6 appeared again in its H_2O component recording a chemical shift at 3.301 ppm for the fresh oil and at 3.312 ppm for the stored oil.

It was noticed an increment in the shift of the second solvent peak to higher value from 3.301 ppm for fresh oil to 3.312 ppm for stored one. This may be attributed to the storage conditions which slightly changed the oil oxidative stability to less stable oil and also because of the more components appeared in the spectra of the stored oil as a result of the slight changes upon storage. These delayed the appearance of the solvent peak and shifted it from 3.301 to 3.312 ppm.

As shown in **Figure (10) and Table (29)**, additional peaks were appeared in the ^{1}H-NMR spectrum of olive oil after 6 months of storage compared with the fresh oil **(Figure 6 and Table 24)**. The most important one was at 2.255 ppm assigned to $–OCO\text{-}CH_2–$ and attributed to the free fatty acids which increased with storage time due to hydrolytic degradation of triglycerides, therefore, the acidity of the oil increased, which means a deterioration of the oil quality. Several resonances disclosed the occurrence of hydrolytic degradation.

In the ^{1}H-NMR spectrum of moringa oil after storage for 6 months **(Figure 11, Table 30)**, a peak assigned for $-CH_3$ (C18-steroid group) and attributed to stigmasterol was appeared at 0.652 ppm with a lower shift than the fresh oil (0.662 ppm). Another peak was appeared at 0.777 ppm shifted from 0.784 ppm in the ^{1}H-NMR spectra of the fresh moringa oil assigned for $-CH_3$ (C18-steroid group) and attributed to β-sitosterol. The peaks assigned for $-CH_3$ (acyl group) which attributed to saturated fatty acids, oleic and linoleic acids were appeared with lower shifts at 0.815, 0.837, 0.859, 0.879, 0.936 and 0.958 ppm compared to 0.843, 0.866, 0.942 and 0.964 ppm assignments of fresh oil. Peaks assigned for $-(CH_2)_n-$ (acyl group) and attributed to aliphatic chain were appeared at lower values of 1.035, 1.058, 1.082, 1.171, 1.241, 1.399 and 1.475 ppm compared to 1.073, 1.185, 1.248, 1.406 and 1.481 ppm for MO before storage. Signals appeared at 1.593 and 1.680 ppm assigned for CH_2-CH_2-COOR and attributed to fatty acids. A peak at 1.702 ppm was appeared with also a shift to a lower value compared with the fresh oil (1.794 ppm) which assigned for $>C=C–CH_3$ (allylic group) and attributed to allyl chain. The peaks representing unsaturated fatty acids and assigned for $–CH_2–CH=CH–$ (acyl group) were appeared with lower and higher shifts at 1.897, 1.970, 1.990, 2.111 and 2.134 ppm compared to the signals at 1.794, 1.925, 2.017 and 2.144 ppm for fresh MO.

New peak was appeared at 2.302 ppm assigned for $–OCO–CH_2–$ and attributed to free fatty acids indicating some slight changes due to hydrolysis and auto-oxidation after storage for 6 months at room temperature. The Peaks appeared at 7.364 and 7.365 ppm which assigned for $–CH=CH–$ and attributed to olefins, were shifted with lower values as compared to 7.370 ppm for the former fresh oil.

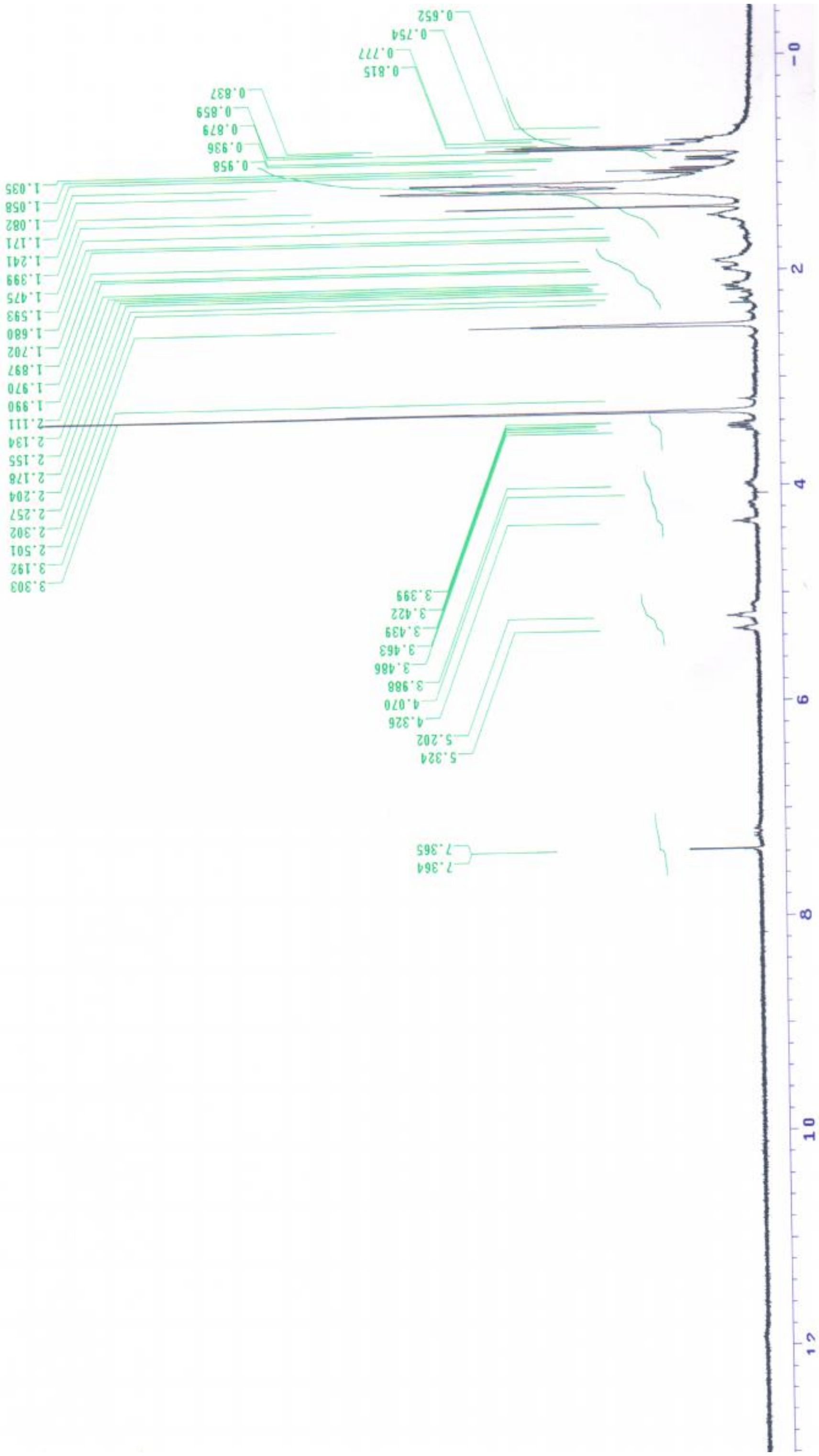


Figure (11) ^{1}H-NMR spectrum of MO after storage for 6 months

Table (30) Chemical shift (δ) assignment of ^{1}H-NMR signals of the main components of MO after storage for 6 months at room temperature in comparison to the chemical shift of the fresh oil

δ (ppm)		Functional group	Assignment
Fresh oil	**After 6 months**		
0.662	0.652	$-CH_3$ (C18-steroid group)	stigmasterol
0.748	0.777	$-CH_3$ (C18-steroid group)	β-sitosterol
	0.815	$-CH_3$ (acyl group)	sat., oleic and linoleic
0.843	0.837	$-CH_3$ (acyl group)	sat., oleic and linoleic
0.866	0.859	$-CH_3$ (acyl group)	sat., oleic and linoleic
	0.879	$-CH_3$ (acyl group)	sat., oleic and linoleic
0.943	0.936	$-CH_3$ (acyl group)	sat., oleic and linoleic
0.964	0.958	$-CH_3$ (acyl group)	sat., oleic and linoleic
	1.035	$-(CH_2)_n-$ (acyl group)	aliphatic chains
1.073	1.058	$-(CH_2)_n-$ (acyl group)	aliphatic chains
1.185	1.171	$-(CH_2)_n-$ (acyl group)	aliphatic chains
1.248	1.241	$-(CH_2)_n-$ (acyl group)	aliphatic chains
1.406	1.399	$-(CH_2)_n-$ (acyl group)	aliphatic chains
1.481	1.475	$-(CH_2)_n-$ (acyl group)	aliphatic chains
1.794	1.702	$>C=C-CH_3$ (allylic group)	allyl chain
	1.897	$-CH_2-CH=CH-$ (acyl group)	unsaturated fatty acids
	1.970	$-CH_2-CH=CH-$ (acyl group)	unsaturated fatty acids
1.925	1.990	$-CH_2-CH=CH-$ (acyl group)	unsaturated fatty acids
2.017	2.111	$-CH_2-CH=CH-$ (acyl group)	unsaturated fatty acids
2.144	2.134	$-CH_2-CH=CH-$ (acyl group)	unsaturated fatty acids
2.262		$-OCO\text{-}CH_2-$ (acyl group)	oleyl acyl chains
2.306		$-OCO\text{-}CH_2-$ (acyl group)	oleyl acyl chains
	2.302	$-OCO\text{-}CH_2-$ (acyl group)	free fatty acids
2.505	2.501	DMSO-d6	solvent DMSO-d6
3.278	3.303	DMSO-d6	H_2O of DMSO –d6
3.875		CH_2OH (glycerol group)	*sn*-1,2 diacylglycerol
4.022	4.070	$>CH-OH$	*sn*-1,3 diacylglycerol
5.095		$-CH_2OCOR$ (glyceryl group)	*sn*-1,3 diacylglycerol
5.138	5.202	$>CHOCOR$ (glyceryl group)	triglycerides
5.215		$>CHOCOR$ (glyceryl group)	triglycerides
5.327	5.324	$-CH=CH-$ (acyl group)	unsaturated fatty acids
7.327	7.364	$-CH=CH-$ (olefinic protons)	olefins
	7.365	$-CH=CH-$ (olefinic protons)	olefins

The ^{1}H-NMR spectrum of apricot kernel oil after storage for 6 months is shown in **(Figure 12 and Table 31)**. A new peak was appeared at 12.200 ppm assigned for hydroxyl group which was not present in the fresh samples of this oil. It was noticed that the signals attributed to saturated fatty acids, oleic, linoleic acids were appeared with lower shifts of 0.763, 0.808, 0.832 and 0.855 ppm assigned for $-CH_3$ (acyl group) in stored AKO, compared to the values of 0.809, 0.819, 0.840 and 0.861 ppm before storage. Also, some shifts of other peaks to a lower values were noticed and the disappearance of the peak at 0.880 ppm and appearance of a peak at 0.933 ppm shifted from 0.939 ppm, all these peaks were assigned for $-CH_3$ (acyl group) and attributed to saturated fatty acids, oleic and linoleic acids. A peak at 0.954 shifted from 0.960 ppm assigned for $-CH_3$ (acyl group) and attributed to the presence of linolenic acid was also found.

The rest of peaks in ^{1}H-NMR spectrum of AKO after 6 months were appeared around nearly the same chemical shifts before storage with shifts to a lower values than in the case of the fresh oil. These peaks were appeared at 1.244, 1.394, 1.470 ppm in stored AKO and shifted from 1.252, 1.401, 1.478 ppm, respectively and assigned to $-(CH_2)_n$ (acyl group) which attributed to aliphatic chains. In addition, there were peaks appeared at 1.967, 2.119 ppm after 6 months were shifted from 2.008, 2.179 ppm, respectively assigned for $-CH_2-CH=CH-$ (acyl group) and attributed to unsaturated fatty acids.

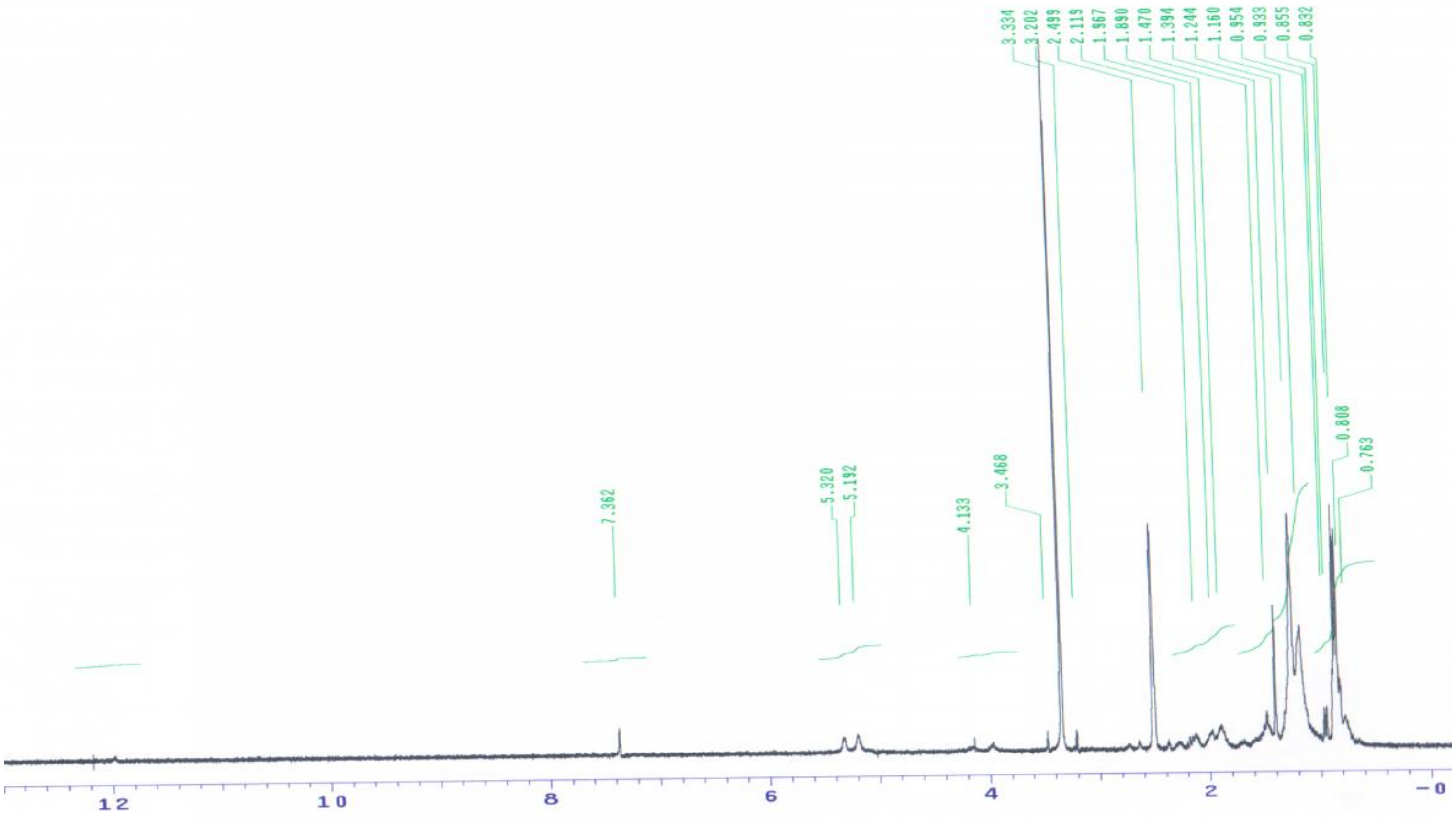


Figure (12) ^{1}H-NMR spectrum of AKO after storage for 6 months

Table (31) Chemical shift (δ) assignment of ^{1}H-NMR signals of the main components of AKO after storage for 6 months at room temperature in comparison to the chemical shift of the fresh oil

δ (ppm)		**Functional group**	**Assignment**
Fresh oil	**After 6 months**		
0.809	0.763	$-CH_3$ (acyl group)	sat., oleic and linoleic
0.819	0.808	$-CH_3$ (acyl group)	sat., oleic and linoleic
0.840	0.832	$-CH_3$ (acyl group)	sat., oleic and linoleic
0.861	0.855	$-CH_3$ (acyl group)	sat., oleic and linoleic
0.880		$-CH_3$ (acyl group)	sat., oleic and linoleic
0.939	0.933	$-CH_3$ (acyl group)	sat., oleic and linoleic
0.960	0.954	$-CH_3$ (acyl group)	linolenic
	1.160	$-(CH_2)_n-$ (acyl group)	aliphatic chains
1.252	1.244	$-(CH_2)_n-$ (acyl group)	aliphatic chains
1.401	1.394	$-(CH_2)_n-$ (acyl group)	aliphatic chains
1.478	1.470	$-(CH_2)_n-$ (acyl group)	aliphatic chains
	1.890	$-CH_2-CH=CH-$ (acyl group)	unsaturated fatty acids
2.008	1.967	$-CH_2-CH=CH-$ (acyl group)	unsaturated fatty acids
2.179	2.119	$-CH_2-CH=CH-$ (acyl group)	unsaturated fatty acids
2.303		$-OCO-CH_2-$ (acyl group)	oleyl acyl chains
2.497	2.499	DMSO-d6	solvent DMSO-d6
2.503		DMSO-d6	solvent DMSO-d6
2.508		DMSO-d6	solvent DMSO-d6
2.739	3.202	$=CH-CH_2-CH=$ (acyl group)	Polyunsaturated fatty acids
3.309	3.334	DMSO-d6	H_2O of DMSO-d6
4.245	3.468	$-CH_2OCOR$ (glyceryl group)	*sn*-1,3 diacylglycerol
4.901	4.133	$-CH_2OCOR$ (glyceryl group)	*sn*-1,3 diacylglycerol
	5.192	$-CH=CH-$ (acyl group)	unsaturated fatty acids
5.327	5.320	$-CH=CH-$ (acyl group)	unsaturated fatty acids
7.368	7.362	$-CH=CH-$ (olefinic protons)	olefins

It was noticed from the obtained results of AKO spectrum after 6 months it was noticed the disappearance of the peaks at 2.497, 2.503 and 2.508 ppm in the ^{1}H-NMR spectra of AKO before storage, and the appearance of a peak at 2.499 ppm assigned for DMSO-d6 and attributed to DMSO-d6 solvent, and showed another peak at 3.334 ppm shifted to a higher value from 3.309 ppm present in fresh AKO spectrum which assigned for DMSO-d6 and attributed to H_2O of DMSO-d6 solvent. It was also noticed the disappearance of a peak at 2.303 ppm assigned for $–OCO–CH_2$ (acyl group) and attributed to oleyl acyl chains that was found in the fresh oil. One peak was appeared at 3.202 ppm assigned for $=CH–CH_2–CH=$ (acyl group) and attributed to polyunsaturated fatty acids. Two peaks were also appeared at 3.468, 4.133 ppm in stored oil which shifted to their lower values from 4.245, 4.901 ppm assigned for $–CH_2OCOR$ (glyceryl group) and attributed to *sn*-1,3 diacylglycerol. In addition, two peaks were appeared at 5.192 ppm shifted from 5.327 ppm along with a peak at 5.320 ppm, these two peaks were assigned for –CH=CH– (acyl group) and attributed to unsaturated fatty acids. Finally, a peak at 7.362 ppm shifted from 7.368 ppm was appeared in stored oil, assigned for –CH=CH– (olefinic protons) and attributed to olefins.

From the aforementioned data, it could be observed that there were some differences between ^{1}H-NMR spectrum of the fresh apricot kernel oil and the stored ones including the disappearance of some peaks and appearance of new peaks. Also, some shifts were denoted in the spectra of the stored oil to lower values than the corresponding chemical shifts before storage. Also a shift to higher value was recorded for one peak representing the water of the solvent.

The ^{1}H-NMR spectrum of sunflower oil after storage for 6 months **(Figure 13, Table 32)**, showed the appearance of a peak at 0.767 ppm as for the fresh oil, while, a new peak was appeared at 0.826 ppm, and another peak at 0.850 ppm in the spectrum of the stored oil that shifted to a lower value from 0.854 ppm before storage, these aforementioned two peaks were assigned for $-CH_3$ (acyl group) and attributed to saturated fatty acids, oleic and linoleic acids. Another peak was appeared at 1.155 ppm in stored SO shifted from the peak at 1.160 ppm assigned for $-(CH_2)_n-$ (acyl group) and attributed to aliphatic chains; along with two new peaks at 1.182 and 1.231 ppm which they were not present in the fresh oil, in addition of a peak at 1.462 ppm shifted from the peak at 1.437 ppm. Three peaks appeared in the ^{1}H-NMR spectra of stored SO, the first one was at 1.912 ppm shifted from 1.896 ppm, the second peak was at 1.994 ppm shifted from 2.106 ppm along with a new additional peak at 2.124 ppm assigned for $–CH_2–CH=CH–$ (acyl group) and attributed to unsaturated fatty acids.

A very important new signal was also appeared at 2.270 ppm assigned for $–OCO\text{-}CH_2–$ and attributed to free fatty acids indicating the effect of hydrolytic degradation of the acyl groups of the oil and the formation of new compounds, as well as conjugated dienic systems. This peak appeared in the stored sunflower oil and was absent in the fresh sunflower spectra. However, two signals were found in the ^{1}H-NMR spectra of fresh SO at 2.492 and 2.497 ppm assigned to $–OCO–CH_2–$ (acyl group) and aroused from oleyl acyl chains; these signals were disappeared in the spectra of the oil after storage for 6 months.

DMSO-d6 peaks after 6 months were appeared at lower shift values of 2.499, 2.504 ppm compared to 2.503 and 2.504 ppm, in addition of a signal at 2.516 ppm appeared in the fresh SO spectrum. Another peak of H_2O of DMSO at higher shift value of 3.339 ppm was found in the spectra of the stored oil shifted from the peak at 3.317 ppm present in the spectrum of fresh SO.

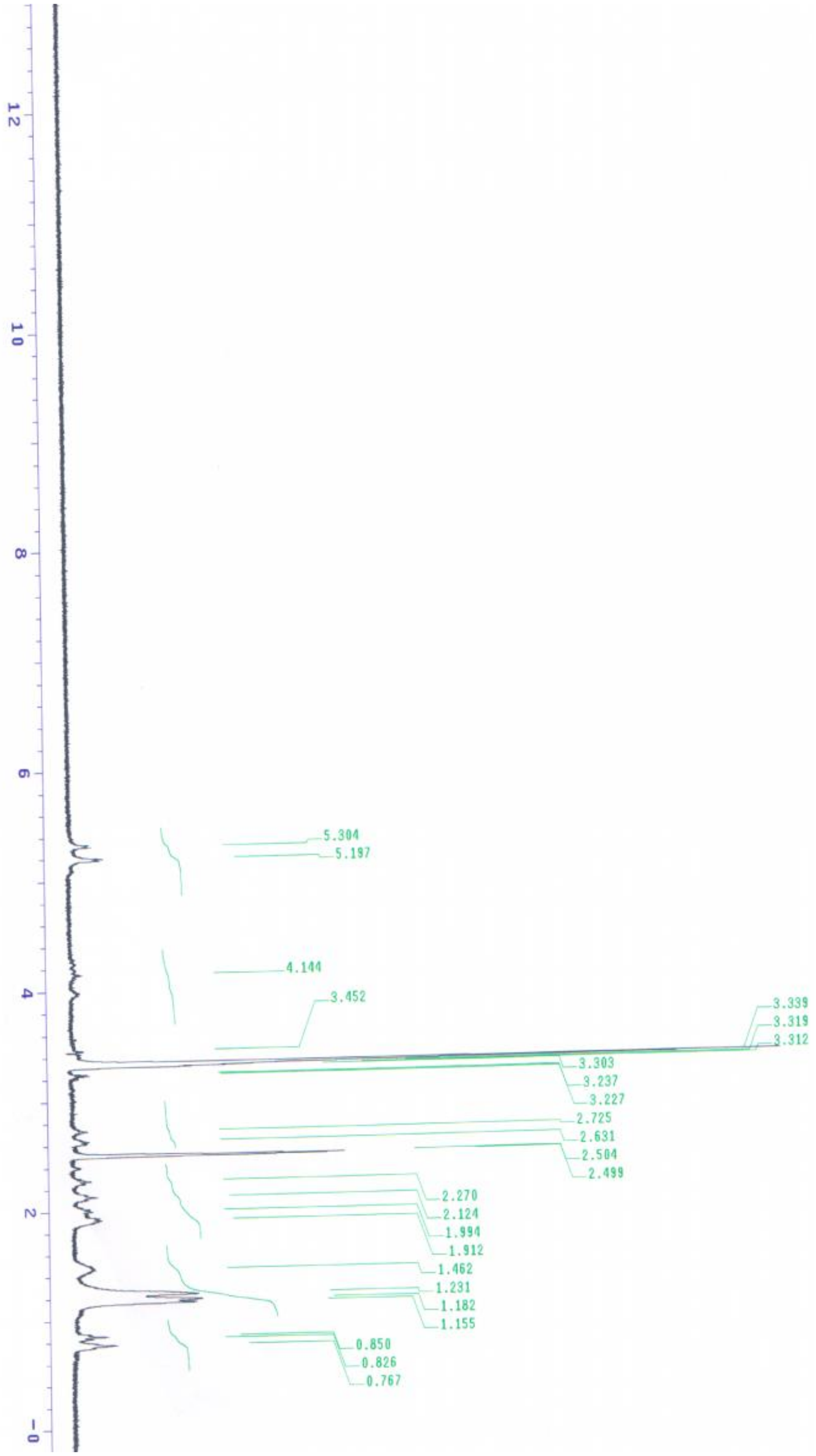


Figure (13) ^{1}H-NMR spectrum of SO after storage for 6 months

Table (32) Chemical shift (δ) assignment of ^{1}H-NMR signals of the main components of SO after storage for 6 months at room temperature in comparison to the chemical shift of the fresh oil

δ (ppm)		**Functional group**	**Assignment**
Fresh oil	**After 6 months**		
0.767	0.767	$-CH_3$ (acyl group)	sat., oleic and linoleic
	0.826	$-CH_3$ (acyl group)	sat., oleic and linoleic
0.854	0.850	$-CH_3$ (acyl group)	sat., oleic and linoleic
1.160	1.155	$-(CH_2)_n-$ (acyl group)	aliphatic chains
	1.182	$-(CH_2)_n-$ (acyl group)	aliphatic chains
	1.231	$-(CH_2)_n-$ (acyl group)	aliphatic chains
1.437	1.462	$-(CH_2)_n-$ (acyl group)	aliphatic chains
1.896	1.912	$–CH_2–CH=CH–$ (acyl group)	unsaturated fatty acids
2.106	1.994	$–CH_2–CH=CH–$ (acyl group)	unsaturated fatty acids
	2.124	$–CH_2–CH=CH–$ (acyl group)	unsaturated fatty acids
2.492		$–OCO–CH_2–$ (acyl group)	oleyl acyl chains
2.497		$–OCO–CH_2–$ (acyl group)	oleyl acyl chains
	2.270	$–OCO–CH_2–$ (acyl group)	free fatty acids
2.503	2.499	DMSO-d6	solvent DMSO-d6
2.510	2.504	DMSO-d6	solvent DMSO-d6
2.516		DMSO-d6	solvent DMSO-d6
2.627	2.631	$-CH_3$ (acyl group)	linoleic
	2.725	$-CH=CH-CH_2-CH=CH-$ (acyl group)	linoleyl and linolenyl
3.317	3.339	DMSO-d6	H_2O of DMSO-d6
3.968		$-CH_2OH-$ (glycerol)	*sn*-1,2 diacylglycerol
4.149	4.144	$–CH_2OCOR$ (glyceryl group)	*sn*-1,3 diacylglycerol
5.184	5.197	$–CH=CH–$ (acyl group)	unsaturated fatty acids
	5.304	$–CH=CH–$ (acyl group)	unsaturated fatty acids

A characteristic peak of linoleic acid assigned to $-CH_3$ (acyl group) was also appeared at 2.631 ppm for stored oil which shifted to a higher value from 2.627 ppm for fresh oil, this peak is a characteristic peak for sunflower oil which has the highest content of linoleic acid, along with a new peak appeared at 2.725 ppm assigned for $-CH=CH-CH_2-CH=CH-$ (acyl group) and attributed to linoleyl and linolenyl moieties. This signal which was at 2.725 ppm aroused from bis-allylic protons from the $–CH_2–$ group located between pairs of unsaturated bonds and thus provide a measure of the number of polyunsaturated fatty acid chains present in the sample. It was well known from the fatty acid composition of sunflower oil discussed earlier, that sunflower oil had a higher value of linoleic acid.

In the ^{1}H-NMR spectrum of stored SO, a peak at 4.144 ppm assigned for $–CH_2OCOR$ (glyceryl group) and attributed to *sn*-1,3 diacylglycerol was appeared with a lower shift from 4.149 ppm in the spectra of the fresh oil. The last two peaks which aroused from unsaturated fatty acids were appeared at higher chemical shifts, the first one was at 5.197 ppm shifted from 5.164 ppm of the fresh oil; while the second peak was a new one and appeared at 5.304 ppm. These two peaks were assigned for $–CH=CH–$ (acyl group).

The degradation of the acyl groups of the oil provokes the formation of new compounds, the ones formed at the 1st step being named primary oxidation compounds. Their natures are varied, and among them some acyl chains supporting hydroperoxy or hydroxy groups, as well as conjugated dienic systems. The possibility that some of the hydroperoxy groups can be supported on saturated and unsaturated acyl chains without conjugated dienic systems exists, as well as the possibility of 2 hydroperoxy groups being supported in the same acyl chain, that is to say the occurrence of di-hydroperoxides in the same acyl chain.

4.3.1.3- Evaluation of Extra Virgin Olive Oil Stability after 60 Months of Storage using ^{1}H-NMR Spectral Data Analysis

As evident in **Figure (14)** and the comparison ^{1}H-NMR spectral data of fresh and stored EVOO for 6 and 60 months in **Table (33)**, some chemical shifts of the ^{1}H-NMR signals of some compounds which appeared in the oil after 6 months, were still unmoved after 60 months of storage such as intensity of β-sitosterol signal at 0.776 ppm with lower value than that of fresh oil (0.780 ppm) as function of $–CH_3$ (C18- steroid group); and the intensity of saturated, oleic and linoleic acids appeared in stored oil for 6 and 6o months, assigned to $–CH_3$ (acyl group) at 0.853 ppm; and the signal at 5.318 ppm assigned to –CH=CH- (acyl group) which attributed to the unsaturated fatty acids with slight higher value compared to fresh EVOO (5.316 ppm); also, the appearance of triglycerides signal assigned to >CHOCOR (glyceryl group) that showed the same intensity at 5.502 ppm in the fresh and stored oil spectra after 60 months with lower value than that of oil stored for 6 months (5.204 ppm).

The ^{1}H-NMR spectra of stored olive oil showed slight changes in intensity of triglycerides and of other component signals compared to those of fresh unstored oil. It was noticed a few lower intensities in the ^{1}H-NMR signals of some compounds after storage such as the intensity of saturated, oleic and linoleic assigned to $–CH_3$ (acyl group) aroused at 0.831 and 0.832 ppm in oil stored for 6 and 60 months, respectively compared to 0.858 ppm of fresh oil; intensity of aliphatic chains assigned to $–(CH_2)_n–$ acyl group at 1.171 ppm after 60 months of storage compared to 1.172 and 1.173 ppm of fresh and stored oil for 6 months, respectively and also at chemical shifts 1.238 and 1.240 ppm in the ^{1}H-NMR signals of stored oils after 6 and 60 months compared to fresh oil (1.242 ppm). A slight lower value was also observed at 1.552 ppm in the stored oil spectra after 60 months attributed to oleyl acyl chains assigned to $–OCO–CH_2–CH_2–$ (acyl group) compared to 1.553 ppm of fresh and stored oil for 6 months having the same value.

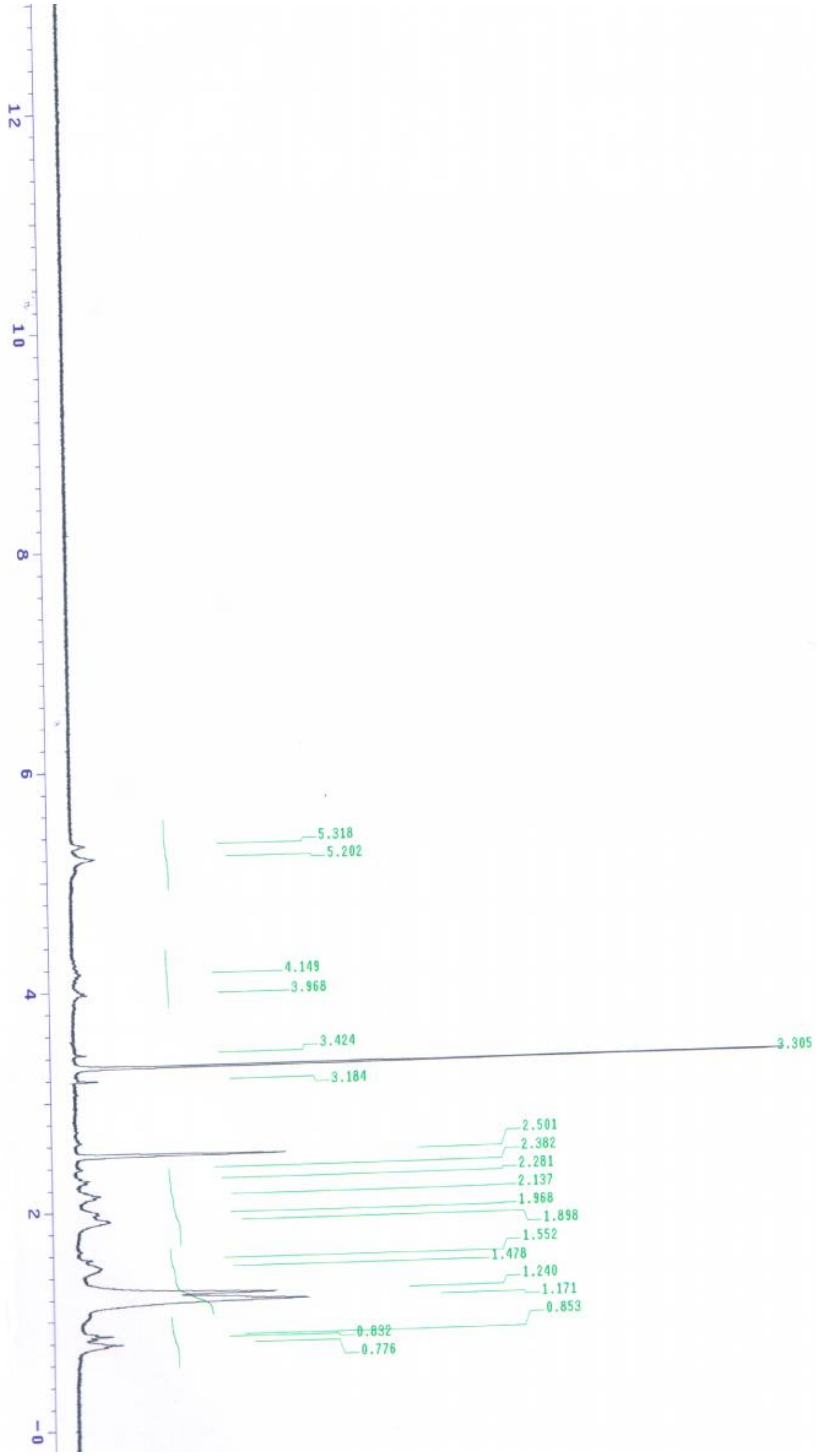


Figure (14) ^{1}H-NMR spectrum of EVOO after storage for 60 months

Table (33) Chemical shift (δ) assignment of ^{1}H-NMR signals of the main components of EVOO after storage for 60 months at room temperature in comparison to the chemical shift of the oil after storage for 6 months and the chemical shift of the fresh oil

δ (ppm)			Functional group	Assignment
Fresh oil	**After 6 months**	**After 60 months**		
0.780	0.776	0.776	$-CH_3$ (C18-steroid group)	β-sitosterol
0.858	0.831	0.832	$-CH_3$ (acyl group)	sat., oleic and linoleic
	0.853	0.853	$-CH_3$ (acyl group)	sat., oleic and linoleic
1.172	1.173	1.171	$-(CH_2)_n-$ (acyl group)	aliphatic chains
1.242	1.238	1.240	$-(CH_2)_n-$ (acyl group)	aliphatic chains
	1.480	1.478	$-(CH_2)_n-$ (acyl group)	aliphatic chains
1.553	1.553	1.552	$-OCO-CH_2-CH_2-$ (acyl group)	oleyl acyl chains
		1.898	$-CH_2-CH=CH-$ (acyl group)	unsaturated fatty acids
	1.967	1.968	$-CH_2-CH=CH-$ (acyl group)	unsaturated fatty acids
	2.136	2.137	$-CH_2-CH=CH-$ (acyl group)	unsaturated fatty acids
	2.255	2.281	$-OCO-CH_2-$	free fatty acids
		2.382	$-OCO-CH_2-$	free fatty acids
2.507			DMSO-d6	solvent DMSO-d6
	2.502	2.501	DMSO-d6	solvent DMSO-d6
2.511			DMSO-d6	solvent DMSO-d6
3.301	3.312	3.305	DMSO-d6	H_2O of DMSO –d6
		3.424	$-CHOH-$	secondary alcohols
		3.968	$-CH_2OH-$	primary alcohols
		4.149	$-CHOH$	1,3-diglycerides
5.202	5.204	5.202	>CHOCOR (glyceryl group)	triglycerides
5.316	5.318	5.318	$-CH=CH-$ (acyl group)	unsaturated fatty acids

From the same **Table (33)**, it is visible the appearance of new signals in the spectra of the stored oils after 6 and 60 months. It was noticed that the chemical shift assignments of these ^{1}H-NMR signals were slightly higher after 60 months than after 6 months of storage. These signals were at 1.968, 2.137 ppm in oil stored for 60 months attributed to unsaturated fatty acids assigned to $–CH_2–CH=CH–$ (acyl group) with higher values than the oil stored for 6 months being at 1.967 and 2.136, respectively; another signal was appeared at 2.281 ppm assigned to $–OCO–CH_2–$ and attributed to free fatty acids also with higher value than that of oil stored for 6 months (2.255 ppm).

It was also noticed new signals which were only appeared in the oxidized olive oil stored for 60 months at 1.898 ppm assigned to $–CH_2–CH=CH–$ (acyl group) and attributed to unsaturated fatty acids; at 2.382 ppm assigned to $–OCO–CH_2–$ and attributed to free fatty acids; at 3.424 ppm assigned to –CHOH– and attributed to secondary alcohols; at 3.968 ppm assigned to $–CH_2OH–$ and attributed to primary alcohols and at 4.149 ppm assigned to –CHOH– and attributed to 1,3 diglycerides. These new signals were aroused as a result of the different oxidative compounds and functional groups which can be presented in oxidized oil. Among these compounds, the different kinds of alcohols, the free fatty acids and diglycerides which were resulted from the oxidation and degradation of the primary oxidative products. These compounds varied in their structure having protons whose signals in the ^{1}H-NMR spectra did not overlap with any other, making it possible to identify. These observations are agreed with **Martínez-Yusta and Guillén (2014).**

The different intensity of the common signals in all spectra given, and the presence or absence of others, allows an initial characterization of oils by simple and direct observation.

Combining the results of fatty acid analysis and evaluation of the oxidative stability of oils by the common Rancimat method, it could be mentioned that in general, the lower the content in polyunsaturated groups in the oil, the higher its resistance to degradation; however, other factors, such as the presence of minor antioxidant components, play a very important role in the degradation rate of the oils, including when the oil is submitted to high temperatures.

As mentioned, this spectroscopic technique is able to study the stability of food lipids as a whole, before and after submission to any degradative process. For this reason, if the information provided is sound, it will be a very useful technique. Several studies have shown the usefulness of this technique in the characterization of non-oxidized food lipids. Nowadays, due to several well-received contributions, the assignment of the signals of ^{1}H-NMR spectra to the protons of the main non-oxidized food lipid components, namely of triglycerides, and also of some minor food lipid components, is well established.

Triglycerides as main oil components underwent changes as a consequence of different kinds of reactions. Some of these reactions only affect the chains of their acyl groups with the triglyceride structure remaining intact. Among these which can be cited: reactions that modify the acyl group chains by incorporation of new additional oxygenated functional groups (hydroperoxy, hydroxyl, epoxy, etc.) or of new unsaturations patterns (conjugated dienic or trienic systems) leading to intermediate or final compounds; reactions that bring about the breaking of acyl group chains leading to the formation of both small molecules exhibiting different functional groups and triglycerides having truncated acyl groups; and polymerization reactions that, modify the acyl group chains by disappearance of double bonds, forming dimers, oligomers or polymers of intact or of modified triglycerides. The occurrence of all these reactions has as its consequence the modification of the composition pattern of the acyl groups.

Structure of fatty acids impacts the chemical reactions happened in TAGs during auto-oxidation. The number and position of C=C are the most important structural factors. Oxidation occurred in the place of C=C is the important and easy-to-happen reaction during the auto-oxidation course. Oxidized triacylglyceride (TAG) monomers involve many classes of variations which are originated from hydroperoxides with extra oxygen. These compounds mainly include the oxidized TAG monomers with keto, epoxy, hydroxyl and aldehyde groups supporting the modified ester acyl chains linked to the backbone of glycerol.

In the end, the ^{1}H nuclear magnetic resonance spectroscopy is a very useful technique in the study of several aspects of edible oils and fats. It has the advantages that it is not destructive, requires a very small sample whose preparation is very simple, and it takes little time.

4.3.2- Evaluation of Oils Stability using ^{13}C-NMR Spectral Data Analysis

The chemical shifts of carbon-13 atoms follows the same shielding and deshielding characteristics that are found in proton NMR spectra; the major exception is that the ring current effects observed for aromatic protons are not observed for aromatic carbons. The hybridization of a carbon determines to a great extent the range within which its ^{13}C-NMR signal is found. The ^{13}C resonances of sp^3 carbon atoms absorb at highest field (-20 to 100 ppm), followed by *sp* carbon atoms (70 to 130 ppm), while sp^2 hybridized centers are shifted farthest (120 to 240 ppm) in organic compounds.

Deuterated dimethyl sulphoxide (DMSO-d6) was used in this investigation as a solvent for the ^{13}C-NMR analysis of the studied oils.

The results obtained from the ^{13}C-NMR spectroscopy of MO and AKO dissolved in DMSO-d6 solvent are shown in **Figures 15, 16** and **Tables (34, 35)**, respectively. However, no data were obtained from the ^{13}C-NMR spectroscopy of EVOO and SO dissolved in DMSO-d6.

It was noticed the apparent peak of DMSO-d6 solvent around 39.51 ppm where it appeared as septet (7 peaks) multiplicity at chemical shifts of 38.660, 38.937, 39.218, 39.495, 39.772, 40.053 and 40.330 ppm for MO; at 38.660, 38.937, 39.218, 39.495, 39.772, 40.049 and 40.334 ppm for AKO. As shown, the DMSO-d6 solvent occupied already all the spectra except a very few peaks which were appeared at 21.996, 26.508

and 28.486 ppm in the aliphatic region for MO (**Figure 15** and **Table 34**) and assigned to C17 (all acylglycerols) LN; C14 (MAG, DAG, TAG) (LO); and C4 (all acylglycerols) OL, LO, LN, respectively. Also, one peak was appeared in the glyceryl region at 69.183 ppm assigned to C2′ (TAG). However, for AKO (**Figure 16** and **Table 35**), there were only two peaks in the aliphatic region appeared at 26.512 and 28.440 ppm assigned for C14 (MAG, DAG, TAG) (LO) and C4 (all acylglycerols) OL, LO, LN, respectively, with only one peak in the glyceryl region at 65.953 ppm assigned for C3′ (1-MAG).

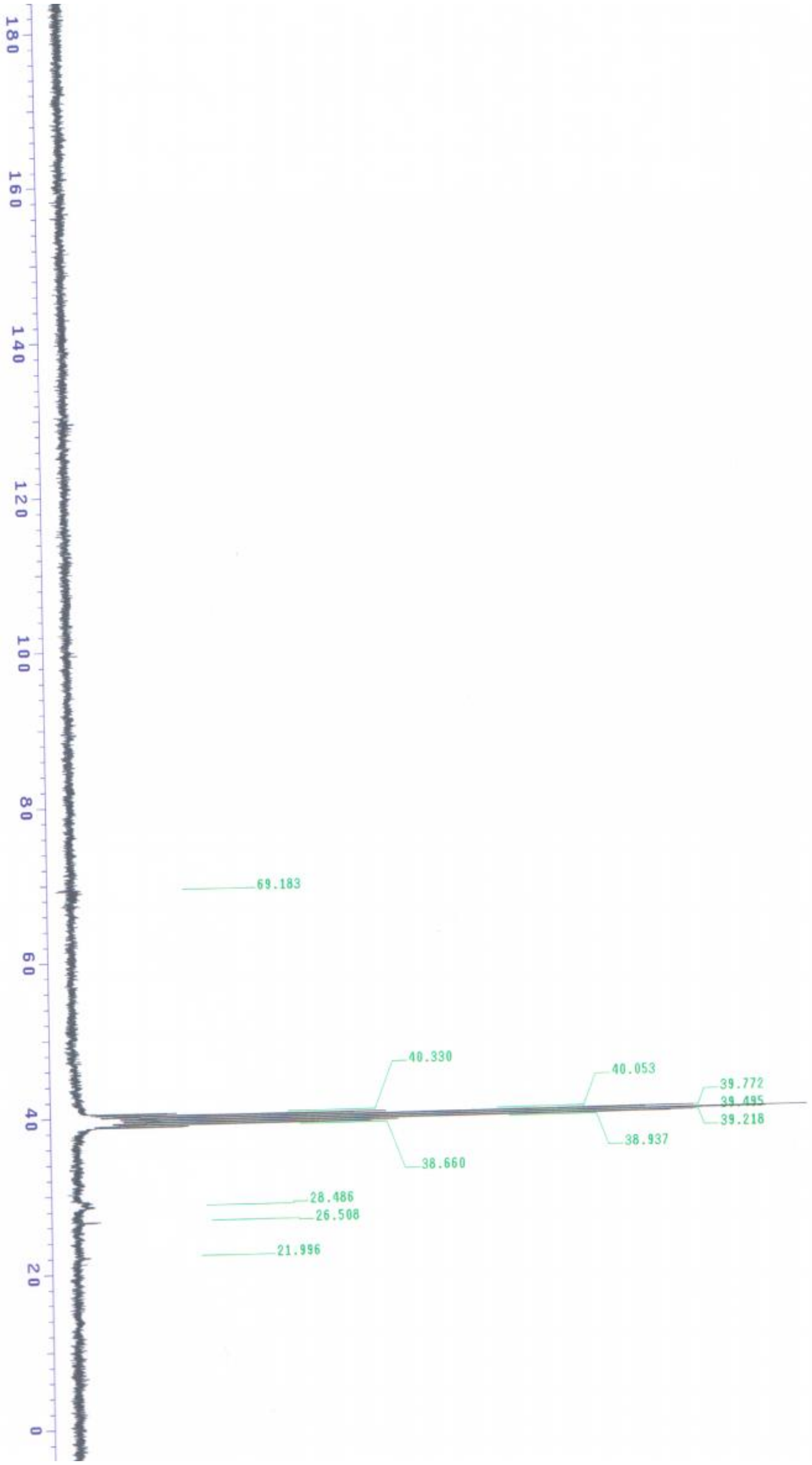


Figure (15) ^{13}C-NMR spectrum of fresh MO in DMSO-d6

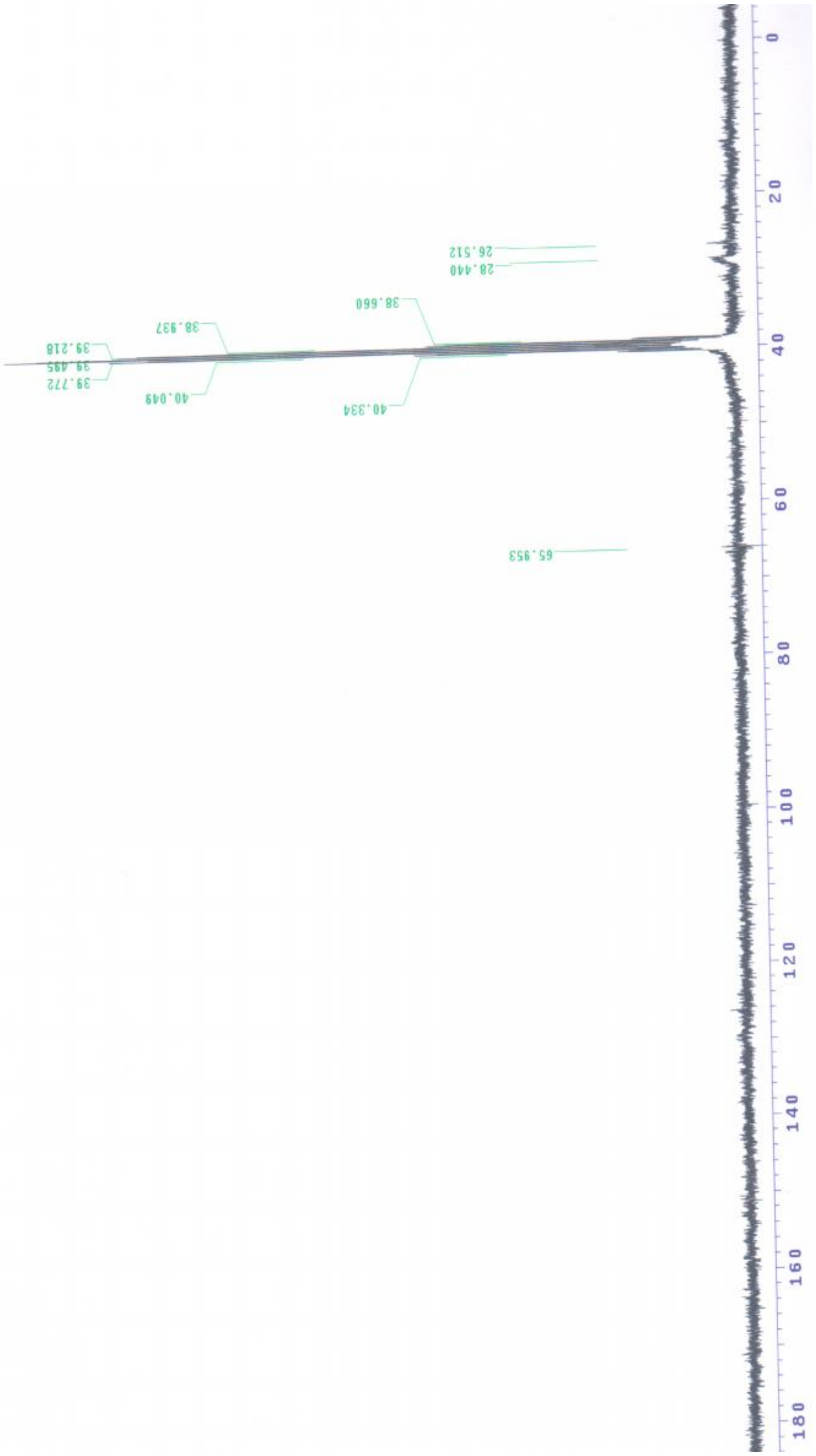


Figure (16) ^{13}C-NMR spectrum of fresh AKO in DMSO-d6

Table (34) Chemical shift (δ) assignment of ^{13}C-NMR signals of the main components of fresh MO in DMSO-d6

δ (ppm)	**Assignment**
21.996	C17 (all acylglycerols) LN
26.508	C14 (MAG, DAG, TAG) (LO)
28.486	C4 (all acylglycerols) OL, LO, LN
38.660	DMSO-d6 solvent
38.937	DMSO-d6 solvent
39.218	DMSO-d6 solvent
39.495	DMSO-d6 solvent
39.772	DMSO-d6 solvent
40.053	DMSO-d6 solvent
40.330	DMSO-d6 solvent
69.183	C2′ (TAG)

Table (35) Chemical shift (δ) assignment of ^{13}C-NMR signals of the main components of fresh AKO in DMSO-d6

δ (ppm)	**Assignment**
26.512	C14 (MAG, DAG, TAG) (LO)
28.440	C4 (all acylglycerols) OL, LO, LN
38.660	DMSO-d6 solvent
38.937	DMSO-d6 solvent
39.218	DMSO-d6 solvent
39.495	DMSO-d6 solvent
39.772	DMSO-d6 solvent
40.049	DMSO-d6 solvent
40.334	DMSO-d6 solvent
65.953	C3′ (1-MAG)

Unfortunately, the ^{13}C-NMR spectra of MO and AKO were very poor in data and were not serviceable for oils analysis when DMSO-d6 solvent was used, where there were no significant peaks appeared in the four regions of spectra; aliphatic, glyceryl, olefinic and carbonyl carbons regions. Thus, the use of DMSO-d6 was not suited as a solvent for the ^{13}C-NMR analysis of the studied oils. Therefore, another trial was carried out using the deuterated chloroform ($CDCl_3$) as NMR solvent in ^{13}C-NMR analysis of the oils.

As shown in **Figures (17-20)** and **Tables (36-39)**, the ^{13}C-NMR spectra of oils under investigation dissolved in $CDCl_3$, a large number of signals were spread over a wide range of chemical shifts. This made the spectra appear complicated but nevertheless was far more informative than the ^{1}H-NMR spectra, which extends to a narrow region of few ppm. Major resonances in the ^{13}C spectra of oil samples were belong to TAG molecules. Four spectral regions were observed in the ^{13}C spectra that belong to four different groups of carbon resonances. The first region was ranging from 172.894-173.334 ppm contained the carbonyl resonances of fatty acids; the second region was from 127.858-130.280 ppm involved resonances of the olefinic carbons of the unsaturated fatty acids; the signals of glycerol backbone carbons appeared in the third region between 62.175 and 69.104 ppm; and the fourth region was from 14.100 to 34.270 ppm comprised signals of aliphatic carbons.

These four regions obtained from the ^{13}C-NMR spectroscopy of the oils under study were agreed with **Zamora *et al.* (2001); Hidalgo and Zamora (2003); Brescia and Sacco (2006); Hatzakis *et al.* (2011); Kumar *et al.* (2011); Dais and Hatzakis (2013).** They mentioned that the four regions of ^{13}C-NMR spectrum of an edible oil were from 172-174; 124-134; 60-72; 10-35 ppm, respectively.

It was noticed from the ^{13}C-NMR spectra of the studied oils, that the sole carbon deuterated chloroform as a solvent showed a triplet chemical shifts around 77 ppm (76.679-77.525) with three peaks being about equal size.

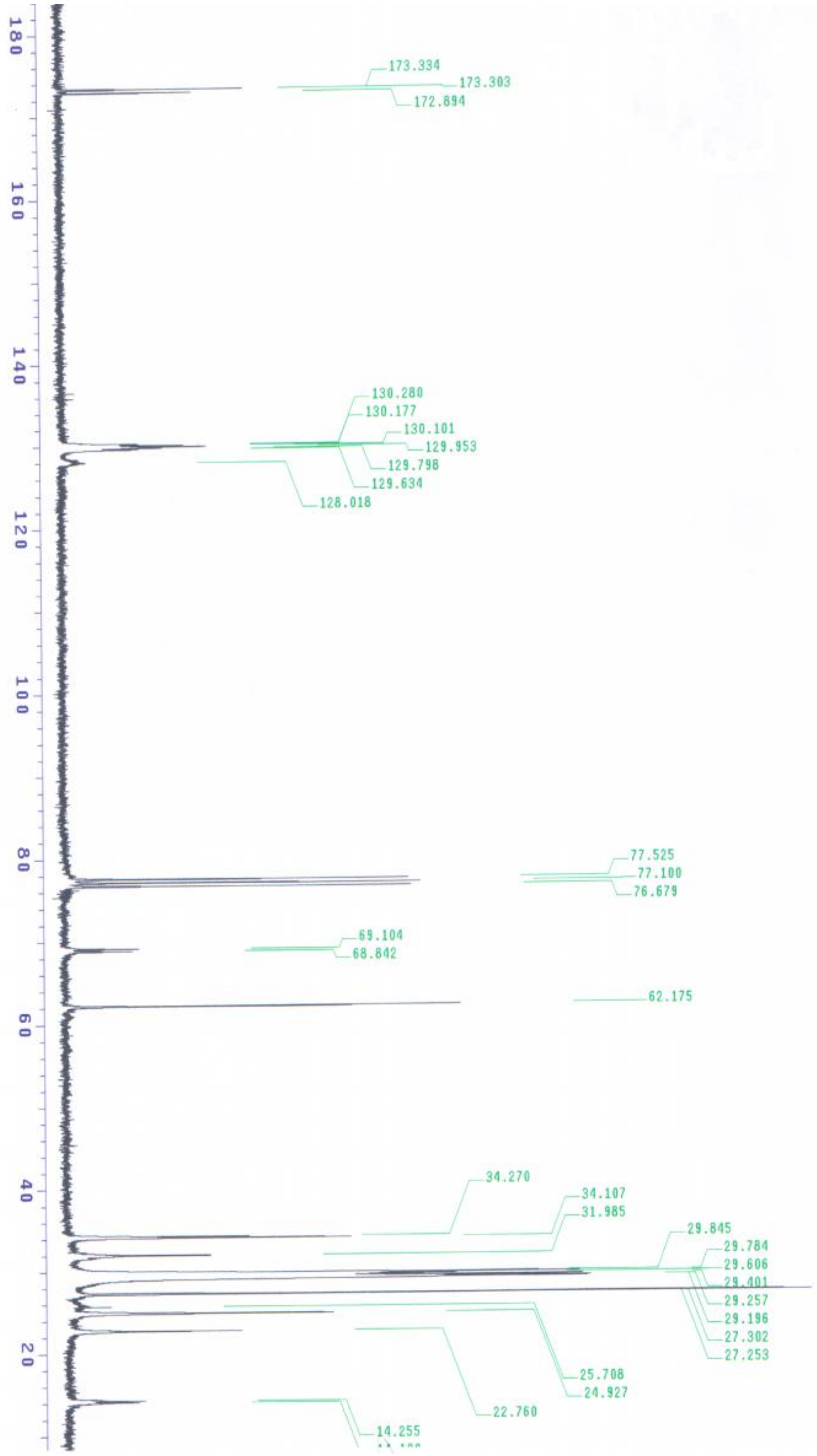


Figure (17) ^{13}C-NMR spectrum of fresh EVOO in $CDCl_3$

Table (36) Chemical shift (δ) assignment of ^{13}C-NMR signals of the main components of fresh EVOO in $CDCl_3$

δ (ppm)	**Assignment & Attribution**
14.100	C18 (all acylglycerols) OL
14.255	C18 (all acylglycerols) LN
22.760	C17 (all acylglycerols) OL, SFA
24.927	C3 (all acylglycerols) SFA
25.708	C11 (all acylglycerols) OL, LO, LN
27.253	C8 (all acylglycerols) LN
27.302	C8 (all acylglycerols) LN
29.196	C6, C15 (all acylglycerols) LO, LN
29.257	C6, C15 (all acylglycerols) LO, LN
29.401	C6 (all acylglycerols) OL, SFA
29.606	C12 (all acylglycerols) OL
29.784	C12 (all acylglycerols) OL
29.845	C12 (all acylglycerols) OL
31.985	C16 (all acylglycerols) SFA
34.107	C2 (MAG, 1,2-DAG) OL, LO, LN
34.270	C2 (MAG, 1,2-DAG) OL, LO, LN
62.175	C1′ (TAG)
68.842	C2′ (TAG)
69.104	C2′ (TAG)
76.679	$CDCl_3$ (Solvent)
77.100	$CDCl_3$ (Solvent)
77.525	$CDCl_3$ (Solvent)
128.018	C10 (DAG, TAG) LO
129.634	C10 (all acylglycerols) OL,
129.798	C9 (all acylglycerols) LO,
129.953	C9 (all acylglycerols) OL
130.101	C13 (DAG, TAG) (LO), C9 (DAG, TAG) (LN)
130.177	C13 (DAG, TAG) (LO), C9 (DAG, TAG) (LN)
130.280	C13 (DAG, TAG) (LO), C9 (DAG, TAG) (LN)
172.894	Linoleyl (LO) acyl chains
173.303	Saturates (S)
173.334	Oleyl (OL) acyl chains

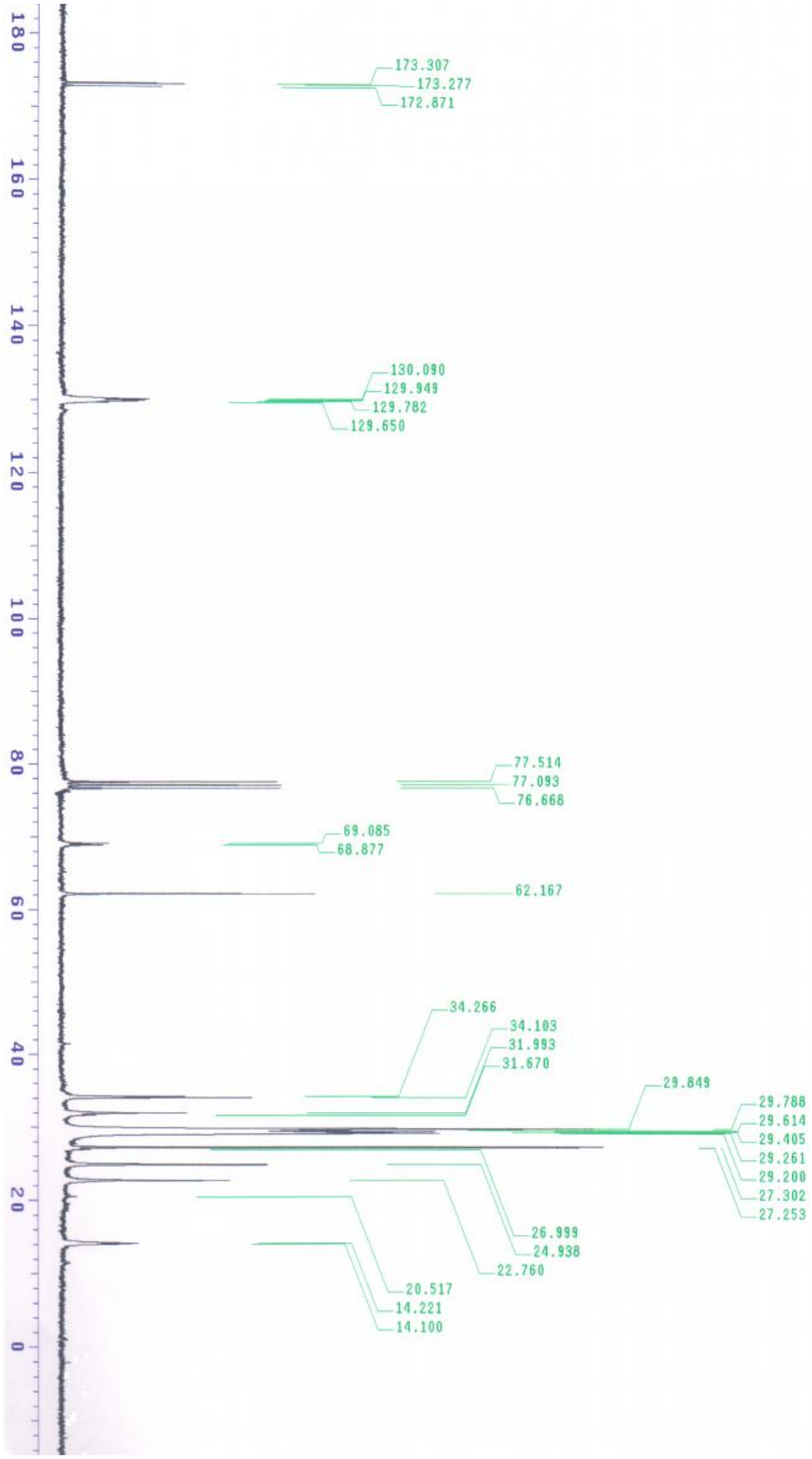


Figure (18) ^{13}C-NMR spectrum of fresh MO in $CDCl_3$

Table (37) Chemical shift (δ) assignment of ^{13}C-NMR signals of the main components of fresh MO in $CDCl_3$

δ (ppm)	**Assignment & Attribution**
14.100	C18 (all acylglycerols) OL
14.221	C18 (all acylglycerols) LN
20.517	C17 (all acylglycerols) LN
22.760	C17 (all acylglycerols) OL, SFA
24.938	C3 (all acylglycerols) SFA
26.999	C14 (MAG, DAG, TAG) (LO)
27.253	C8 (all acylglycerols) LN
27.302	C8 (all acylglycerols) LN
29.200	C6, C15 (all acylglycerols) LO, LN
29.261	C6, C15 (all acylglycerols) LO, LN
29.405	C6 (all acylglycerols) OL, SFA
29.614	C12 (all acylglycerols) OL
29.788	C12 (all acylglycerols) OL
29.849	C12 (all acylglycerols) OL
31.670	C16 (all acylglycerols) SFA
31.993	C16 (all acylglycerols) SFA
34.103	C2 (MAG, 1,2-DAG) OL, LO, LN
34.266	C2 (MAG, 1,2-DAG) OL, LO, LN
62.167	C1′ (TAG)
68.877	C2′ (TAG)
69.085	C2′ (TAG)
76.668	$CDCl_3$ (Solvent)
77.093	$CDCl_3$ (Solvent)
77.514	$CDCl_3$ (Solvent)
129.650	C10 (all acylglycerols) OL,
129.782	C9 (all acylglycerols) LO,
129.949	C9 (all acylglycerols) OL
130.090	C13 (DAG, TAG) (LO), C9 (DAG, TAG) (LN)
172.871	Linoleyl (LO) acyl chains
173.277	Saturates (S)
173.307	Oleyl (OL) acyl chains

Since the components of the triacylglycerol fraction determined by ^{13}C-NMR (or ^{1}H-NMR) consist of 95% of oil total concentration, the unique using ^{13}C-NMR spectroscopy in oil analysis is the ability of this technique to furnish reliable information about the positional distribution of acyl chains on the glycerol backbone. Positions and intensities of the signals were a consequence of the fatty acid profile of the oils and the position of these acyl chains in the triacylglycerols. This could be observed in different parts of the spectra. The carbonyl region alone can be used for this purpose. As mentioned above, this region ranging from 172-174 ppm and will be discussed for each oil as follows.

Each of EVOO and MO contained only saturates (S), oleyl (O), and linoleyl (L) acyl chains represented by the respective peaks at 173.334, 173.303, 172.864 ppm for EVOO; and at 173.307, 173.277, 172.871 ppm for MO. Each of these groups were resolved in the carbonyl region as shown in **Figures (17, 18)** and **Tables (36, 37)** which represent the ^{13}C-NMR spectrum of EVOO and MO, respectively. The carbonyl carbon of linolenyl chain was not detected by ^{13}C-NMR due to the very low concentration (≤0.9%) of this acyl chain in EVOO and MO.

As shown in **Figures (19, 20)** and **Tables (38, 39)**, AKO and SO contained only oleyl (O), and linoleyl (L) acyl chains represented by peaks at 173.303, 172.894 ppm, and at 173.300, 172.890 ppm, respectively. Each of these groups was resolved in the carbonyl region as shown in the ^{13}C-NMR spectrum of AKO and SO. The carbonyl carbon of linolenyl chain was not detected by ^{13}C-NMR due to the very low concentration (≤0.9%) of this acyl chain in AKO and SO. It was noticed the absence of the peak of saturates appeared in EVOO and MO since AKO and SO had low content of SFA being 5.66 and 12.79% compared with EVOO (17.43%) and MO (23.14%) which appeared at 173.334 and 173.307, respectively. This phenomenon will be useful to differentiate AKO and SO from EVOO and MO.

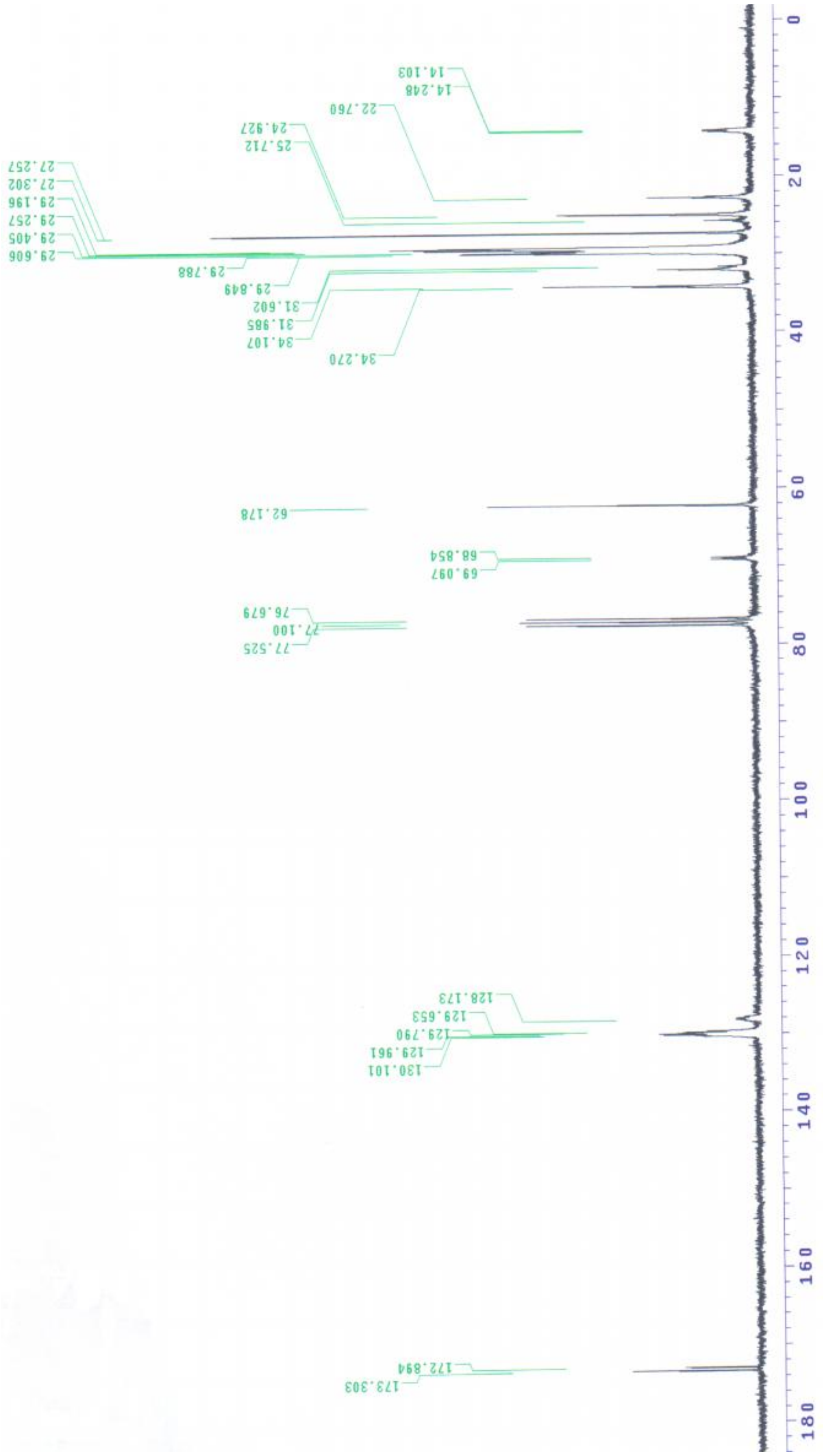


Figure (19) ^{13}C-NMR spectrum of fresh AKO in $CDCl_3$

Table (38) Chemical shift (δ) assignments of ^{13}C-NMR signals of the main components of fresh AKO in $CDCl_3$

δ (ppm)	Assignment & Attribution
14.103	C18 (all acylglycerols) OL
14.248	C18 (all acylglycerols) LN
22.760	C17 (all acylglycerols) OL, SFA
24.927	C3 (all acylglycerols) SFA
25.712	C11 (all acylglycerols) OL, LO, LN
27.257	C8 (all acylglycerols) LN
27.302	C8 (all acylglycerols) LN
29.196	C6, C15 (all acylglycerols) LO, LN
29.257	C6, C15 (all acylglycerols) LO, LN
29.405	C6 (all acylglycerols) OL, SFA
29.606	C12 (all acylglycerols) OL
29.788	C12 (all acylglycerols) OL
29.849	C12 (all acylglycerols) OL
31.602	C16 (all acylglycerols) SFA
31.985	C16 (all acylglycerols) SFA
34.107	C2 (MAG, 1,2-DAG) OL, LO, LN
34.270	C2 (MAG, 1,2-DAG) OL, LO, LN
62.178	C1′ (TAG)
68.854	C2′ (TAG)
69.097	C2′ (TAG)
76.679	$CDCl_3$ (Solvent)
77.100	$CDCl_3$ (Solvent)
77.525	$CDCl_3$ (Solvent)
128.173	C10 (DAG, TAG) LO
129.653	C10 (all acylglycerols) OL,
129.790	C9 (all acylglycerols) LO,
129.961	C9 (all acylglycerols) OL
130.101	C13 (DAG, TAG) (LO), C9 (DAG, TAG) (LN)
172.894	Linoleyl (LO) acyl chains
173.303	Oleyl (OL) acyl chains

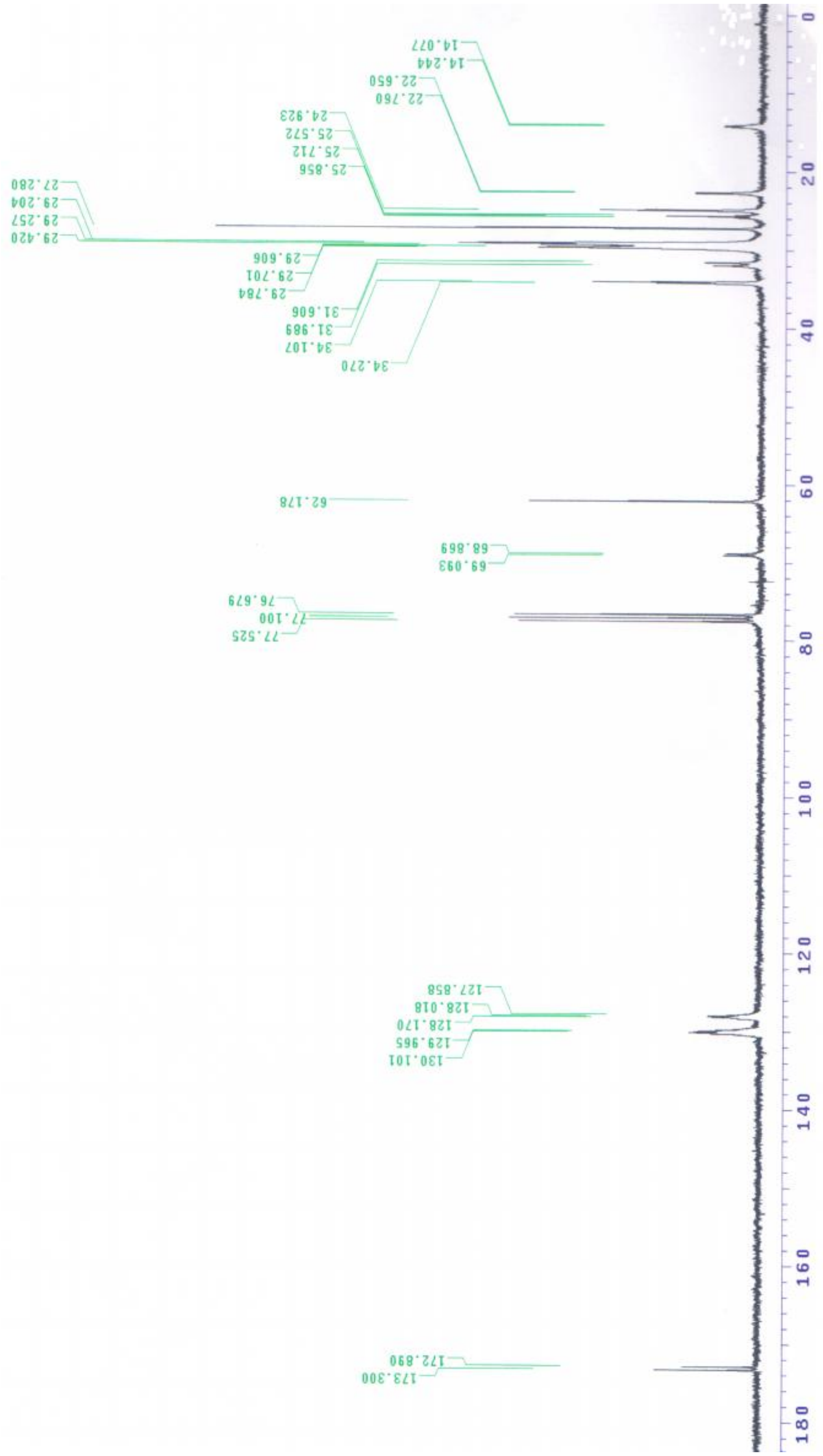


Figure (20) ^{13}C-NMR spectrum of fresh SO in $CDCl_3$

Table (39) Chemical shift (δ) assignment of ^{13}C-NMR signals of the main components of fresh SO in $CDCl_3$

δ (ppm)	**Assignment & Attribution**
14.077	C18 (all acylglycerols) OL
14.244	C18 (all acylglycerols) LN
22.650	C17 (all acylglycerols) OL, SFA
22.760	C17 (all acylglycerols) OL, SFA
24.923	C3 (all acylglycerols) SFA
25.572	C14 (all acylglycerols) LN
25.712	C11 (all acylglycerols) OL, LO, LN
25.856	C11 (all acylglycerols) OL, LO, LN
27.280	C8 (all acylglycerols) LN
29.204	C6, C15 (all acylglycerols) LO, LN
29.257	C6, C15 (all acylglycerols) LO, LN
29.420	C6 (all acylglycerols) OL, SFA
29.606	C12 (all acylglycerols) OL
29.701	C12 (all acylglycerols) OL
29.784	C12 (all acylglycerols) OL
31.606	C16 (all acylglycerols) SFA
31.989	C16 (all acylglycerols) SFA
34.107	C2 (MAG, 1,2-DAG) OL, LO, LN
34.270	C2 (MAG, 1,2-DAG) OL, LO, LN
62.178	C1′ (TAG)
68.869	C2′ (TAG)
69.093	C2′ (TAG)
76.679	$CDCl_3$ (Solvent)
77.100	$CDCl_3$ (Solvent)
77.525	$CDCl_3$ (Solvent)
127.858	C12 (DAG, TAG) LO
128.018	C10 (DAG, TAG) LO
128.170	C10 (DAG, TAG) LO
129.965	C10 (all acylglycerols) OL, C9 (all acylglycerols) LO, C9 (all acylglycerols) OL
130.101	C13 (DAG, TAG) (LO), C9 (DAG, TAG) (LN)
172.890	Linoleyl (LO) acyl chains
173.300	Oleyl (OL) acyl chains

Table (40) Comparison of chemical shift (δ) assignment of ^{13}C-NMR signals of the main components of the fresh studied in $CDCl_3$

δ (ppm)				Assignment & Attribution
EVOO	**MO**	**AKO**	**SO**	
14.100	14.100	14.103	14.077	C18 (all acylglycerols) OL
14.255	14.221	14.248	14.244	C18 (all acylglycerols) LN
	20.517			C17 (all acylglycerols) LN
			22.650	C17 (all acylglycerols) OL, SFA
22.760	22.760	22.760	22.760	C17 (all acylglycerols) OL, SFA
24.927	24.938	24.927	24.923	C3 (all acylglycerols) SFA
			25.572	C14 (all acylglycerols) LN
25.708		25.712	25.712	C11 (all acylglycerols) OL, LO, LN
			25.856	C11 (all acylglycerols) OL, LO, LN
	26.999			C14 (MAG, DAG, TAG) (LO)
27.253	27.253	27.257		C8 (all acylglycerols) LN
27.302	27.302	27.302	27.280	C8 (all acylglycerols) LN
29.196	29.200	29.196	29.204	C6, C15 (all acylglycerols) LO, LN
29.257	29.261	29.257	29.257	C6, C15 (all acylglycerols) LO, LN
29.401	29.405	29.405	29.420	C6 (all acylglycerols) OL, SFA
29.606	29.614	29.606	29.606	C12 (all acylglycerols) OL
			29.701	C12 (all acylglycerols) OL
29.784	29.788	29.788	29.784	C12 (all acylglycerols) OL
29.845	29.849	29.849		C12 (all acylglycerols) OL

Table (40) (continued)

δ (ppm)				Assignment & Attribution
EVOO	**MO**	**AKO**	**SO**	
	31.670	31.602	31.606	C16 (all acylglycerols) SFA
31.985	31.993	31.985	31.989	C16 (all acylglycerols) SFA
34.107	34.103	34.107	34.107	C2 (MAG, 1,2-DAG) OL, LO, LN
34.270	34.266	34.270	34.270	C2 (MAG, 1,2-DAG) OL, LO, LN
62.175	62.167	62.178	62.178	C1′ (TAG)
68.842	68.877	68.854	68.869	C2′ (TAG)
69.104	69.085	69.097	69.093	C2′ (TAG)
76.679	76.668	76.679	76.679	$CDCl_3$ (Solvent)
77.100	77.093	77.100	77.100	$CDCl_3$ (Solvent)
77.525	77.514	77.525	77.525	$CDCl_3$ (Solvent)
			127.858	C12 (DAG, TAG) LO
			128.018	C10 (DAG, TAG) LO
128.018		128.173	128.170	C10 (DAG, TAG) LO
129.634	129.650	129.653		C10 (all acylglycerols) OL,
129.798	129.782	129.790		C9 (all acylglycerols) LO,
129.953	129.949	129.961	129.965	C9 (all acylglycerols) OL
130.101	130.090	130.101	130.101	C13 (DAG, TAG) (LO), C9 (DAG, TAG) (LN)
130.177				C13 (DAG, TAG) (LO), C9 (DAG, TAG) (LN)
130.280				C13 (DAG, TAG) (LO), C9 (DAG, TAG) (LN)
172.894	172.871	172.894	172.890	Linoleyl (LO) acyl chains
173.303	173.277			Saturates (S)
173.334	173.307	173.303	173.300	Oleyl (OL) acyl chains

These obtained results agreed with **Kumar *et al.* (2011)** who revealed that the ^{13}C-NMR spectral analysis provides a clear distinction between the signals of an acid and an ester moiety. The carbonyl signal of free fatty acids appeared between 177.00–182.00 ppm, whereas ester signals in triglycerides resonated at 172.00–174.00 ppm.

In this study, gas chromatography (GC) was used as a recognized analytical technique for fatty acid analysis and thus, it was frequently used to validate NMR analysis results. The obtained data from ^{13}C-NMR were found to be well correlated with gas chromatographic analyses of fatty acids in oils **(Table 5)**. The different studied oils exhibited some differences in quantitative chain composition rather than in qualitative acid profile, therefore exhibiting analogous signals in the ^{13}C NMR spectrum, although with varying intensities which are characteristic for each oil. Positions and intensities of the signals are a consequence of the fatty acid profile of the oils and the position of these acyl chains in the triacylglycerols. This can be observed in different parts of the spectra.

The different olefinic carbons of the different fatty acids and in the different positions of the triacylglycerol molecule appeared at different chemical shifts ranging from 127.858-130.280 ppm in the ^{13}C-NMR spectral data of the studied oils as shown in **Figures (17-20)** and **Tables (36-39)**.

The ^{13}C-NMR spectral data of EVOO **(Figure 17 and Table 36)** showed the resonances of the olefinic carbons of the unsaturated fatty acids represented by peaks generated at 130.280, 130.177, 130.101 ppm assigned to C13 (DAG, TAG) (LO), C9 (DAG, TAG) (LN); at 129.953, 129.798, 129.634 ppm [C10 (all acylglycerols) OL, C9 (all acylglycerols) LO, C9 (all acylglycerols) OL] and at 128.018 ppm assigned to C10 (DAG, TAG) LO.

Concerning MO **(Figure 18 and Table 37),** the olefinic carbon signals of the unsaturated fatty acids appeared by peaks evolved at 130.090 ppm assigned to C13 (DAG, TAG) (LO), C9 (DAG, TAG) (LN); at 129.949, 129.782 ppm and 129.650 ppm attributed to C10 (all acylglycerols) OL, C9 (all acylglycerols) LO, C9 (all acylglycerols) OL, respectively. However, these signals were masked in AKO ^{13}C-NMR spectrum as shown in **Figure (19)** and the data in **Table (38)** by peaks aroused at 130.101 ppm assigned to C13 (DAG, TAG) (LO), C9 (DAG, TAG) (LN); at 129.961, 129.790 and 129.653 ppm assigned to C10 (all acylglycerols) OL, C9 (all acylglycerols) LO, C9 (all acylglycerols) OL, respectively, and 128.173 ppm assigned to C10 (DAG, TAG) LO.

Figure (20) and **Table (39)** also showed the olefinic carbon signals of SO unsaturated fatty acids which emerged by peaks at 130.101 ppm assigned to C13 (DAG, TAG) (LO), C9 (DAG, TAG) (LN); at 129.965 ppm assigned to C10 (all acylglycerols) OL, C9 (all acylglycerols) LO, C9 (all acylglycerols) OL; at 128.170 and 128.018 ppm assigned to C10 (DAG, TAG) LO and at 127.858 ppm assigned to C12 (DAG, TAG) LO.

It was interesting to note that this investigation disclosed the position of the glyceryl backbone carbon of the studied oils through the peaks observed in the third region between 62.175 and 69.104 ppm. The characteristic signal of each functional group like carbon–carbon double bonds, ester, acid and long alkyl chain were assigned. It was clearly noted that the C-1 and C-3 terminal carbons of the triglyceride moiety resonate at the same chemical shift around 62 (62.175-62.178) ppm, whereas C-2 carbons resonate around 68.8 (68.842-69.104) ppm.

As shown in **Figure (17)** and **Table (36)**, EVOO gave carbon signals of the glyceryl backbone of the oil represented by peaks created at 69.104 and 68.842 ppm assigned to C2′ (TAG) and at 62.175 ppm assigned to [C1′ (TAG)]. However, MO **(Figure 18 and Table 37)** induced carbon signals of the glycerol backbone represented by peaks evolved at 69.085 and 68.877 ppm assigned to C2′ (TAG) and at 62.167 ppm assigned to C1′ (TAG). AKO **(Figure 19 and Table 38)** showed these carbon signals aroused by peaks at 69.097 and 68.854 ppm assigned to C2′ (TAG) and at 62.178 ppm assigned to C1′ (TAG). Also, the spectrum of SO **(Figure 20)** and the data in **Table (39)** showed these carbon signals appeared by the peaks at 69.093 and 68.869 ppm assigned to C2′ (TAG) and at 62.178 ppm assigned to C1′ (TAG).

Considering the fourth region from 10 to 35 ppm, which comprises the signals of aliphatic carbons, the ^{13}C-NMR spectrum of EVOO **(Figure 17)** and the data in **Table (36)** showed these carbon signals aroused by peaks at 34.270 and 34.107 ppm assigned to C2 (MAG, 1,2-DAG) OL, LO, LN; at 31.985 ppm assigned to C16 (all acylglycerols) SFA; at 29.845, 29.784 and 29.606 ppm assigned to C12 (all acylglycerols) OL; at 29.401 ppm assigned to C6 (all acylglycerols) OL, SFA; at 29.257 and 29.196 ppm assigned to C6, C15 (all acylglycerols) LO, LN; at 27.302 and 27.253 ppm assigned to C8 (all acylglycerols) LN; at 25.708 ppm assigned to C11 (all acylglycerols) OL, LO, LN; at 24.927 ppm assigned to C3 (all acylglycerols) SFA; at 22.760 ppm assigned to C17 (all acylglycerols) OL, SFA; at 14.255 ppm assigned to C18 (all acylglycerols) LN and at 14.100 ppm assigned to C18 (all acylglycerols) OL.

As shown in **Figure (18)** and **Table (37)**, MO caused the signals of aliphatic carbons represented by peaks at 34.266 ppm assigned to C2 (MAG, 1,2-DAG) OL, LO, LN; at 37.103, 31.993 and 31.670 ppm assigned to C16 (all acylglycerols) SFA; at 29.849, 29.788 and 29.614 ppm assigned to C12 (all acylglycerols) OL; at 29.405 ppm assigned to C6 (all acylglycerols) OL, SFA; at 29.261 and 29.200 ppm assigned to C6, C15 (all acylglycerols) LO, LN; at 27.302 and 27.253 ppm assigned to C8 (all acylglycerols) LN; at 26.999 ppm assigned to C14 (MAG, DAG, TAG) (LO); at 24.938 ppm assigned to C3 (all acylglycerols) SFA; at 22.760 ppm assigned to C17 (all acylglycerols) OL, SFA; at 20.517 ppm assigned to C17 (all acylglycerols) LN; at 14.255 ppm assigned to C18 (all acylglycerols) LN and at 14.100 ppm assigned to C18 (all acylglycerols) OL.

AKO produced aliphatic carbon signals represented by peaks at 34.270 and 34.107 ppm assigned to C2 (MAG, 1,2-DAG) OL, LO, LN; at 31.985 and 31.602 ppm assigned to C16 (all acylglycerols) SFA; at

29.849, 29.788 and 29.606 ppm assigned to C12 (all acylglycerols) OL; at 29.405 ppm assigned to C6 (all acylglycerols) OL, SFA; at 29.257 and 29.196 ppm assigned to C6, C15 (all acylglycerols) LO, LN; at 27.302 and 27.257 ppm assigned to C8 (all acylglycerols) LN; at 25.712 ppm assigned to C11 (all acylglycerols) OL, LO, LN; at 24.927 ppm assigned to C3 (all acylglycerols) SFA; at 22.760 ppm assigned to C17 (all acylglycerols) OL, SFA; at 14.255 ppm assigned to C18 (all acylglycerols) LN and at 14.100 ppm assigned to [C18 (all acylglycerols) OL] **(Figure 19 and Table 38)**.

Also from **Figure (20)** and **Table (39)**, SO gave rise to aliphatic carbon signals represented by peaks at 34.270 and 34.107 ppm assigned to C2 (MAG, 1,2-DAG) OL, LO, LN; at 31.989 and 31.606 ppm assigned to C16 (all acylglycerols) SFA; at 29.784, 29.701 and 29.606 ppm assigned to C12 (all acylglycerols) OL; at 29.420 ppm assigned to C6 (all acylglycerols) OL, SFA; at 29.257 and 29.204 ppm assigned to C6, C15 (all acylglycerols) LO, LN; at 27.280 ppm assigned to C8 (all acylglycerols) LN; at 25.856 and 25.712 ppm assigned to C11 (all acylglycerols) OL, LO, LN; at 25.572 ppm assigned to C14 (all acylglycerols) LN; at 24.923 ppm assigned to C3 (all acylglycerols) SFA; at 22.760 and 22.650 ppm assigned to C17 (all acylglycerols) OL, SFA; at 14.255 ppm assigned to C18 (all acylglycerols) LN and at 14.100 ppm assigned to C18 (all acylglycerols) OL.

In a comparison of the two solvents used in the ^{13}C-NMR spectral analyses of oils under investigation; $CDCl_3$ (deuterated chloroform) and DMSO-d6 (deuterated dimethyl sulphoxide), it could be noticed that all the oils showed excellent spectral figures and data when $CDCl_3$ was used instead of DMSO-d6. This fact is due to the more powerful dissolving effect of $CDCl_3$ much greater than DMSO-d6 as discussed earlier.

Deuterated chloroform ($CDCl_3$) is an organic solvent for suspending the molecules under study in solution, most compounds dissolved in it, it is volatile and therefore easy to get rid of (its boiling point 62°C) and it is non-reactive and will not exchange its deuterium with protons in the molecule being studied. It is also "silent" in the NMR and did not show peaks to interfere with the analysis of the compound of interest. However, DMSO is an organosulfur compound with the formula $(CH_3)_2SO$. Because of its high boiling point, 189°C, it evaporates slowly at normal atmospheric pressure. Samples dissolved in DMSO cannot be as easily recovered. In its deuterated form (DMSO-d6), it is a useful solvent for NMR spectroscopy due to its ability to dissolve a wide range of analytes, the simplicity of its own simple spectrum, and its suitability for high-temperature NMR spectroscopic studies. Disadvantages to the use of DMSO-d6 are its high viscosity, which broadens signals, and its hygroscopicity, which leads to an overwhelming H_2O resonance in the ^{1}H-NMR spectrum.

In this study DMSO-d6 showed its utility as a solvent in the ^{1}H-NMR spectroscopic analysis of the oils, while it was not suitable in the ^{13}C-NMR spectral analysis as mentioned above.

From the obtained results, it could be seen that the ^{13}C-NMR technique is particularly useful in distinguishing between mono-, di- and tri-glycerides, a strong discriminating power to detect the specific components and free fatty acids.

From the discussion of the obtained data shown in **Figures (17-20)** and **Tables (36-39)** and from **Table (40)** which summarized the comparison between ^{13}C-NMR spectroscopy of oils under investigation, it was deduced the discriminating differences between oils under investigations that could be concluded as follows:

The solvent peaks appeared as triplet multiplicity for $CDCl_3$ solvent around 77.100 ppm. It was noticed that MO had the lowest chemical shifts at 77.514, 77.093 and 76.668 ppm, whereas, the rest oils (EVOO, AKO and SO) had the same chemical shifts at 77.525, 77.100 and 76.679 ppm. This may reflect the highest stability of MO among all the oils under investigation.

With regard to the analytical regions which contained the significant data for analysis other than the solvent region, in the first region that ranging from 172-174 ppm and contains the carbonyl resonances of fatty acids, it was observed the absence of peak resonating at 173 ppm characteristic of saturates (S) in AKO and SO, where it appeared in both EVOO and MO at 173.303 and 173.277 ppm, respectively. These findings agreed with the GC analysis of fatty acids, where SFA were the highest in MO (23.14%) followed by EVOO (17.43%) then SO (12.79) and at last AKO (5.66%).

In the second region ranging from 124-134 ppm which involves the resonances of the olefinic carbons of the unsaturated fatty acids, it was noticed the presence of two peaks at 127.858 and 128.018 ppm assigned to C12 (DAG, TAG) LO and C10 (DAG, TAG) LO, respectively, which were shown only in SO and were absent in the other oils. These spectral data agreed with the GC analysis of fatty acids, where linoleic acid (LO) was the major fatty acid in SO (52.2%) that appeared clearly in ^{13}C-NMR spectra of SO.

It was noticed the absence of two peaks at 129.650 and 129.790 ppm assigned for C10 (all acylglycerols) OL and C9 (all acylglycerols) LO, respectively in SO, but they were present in the other oils (EVOO, MO and AKO) indicating that these three oils had higher content of oleic acid (OL) and lower content of linoleic acid (LO) than had SO which was higher in linoleic acid (LO) and lower in oleic acid (OL). These results go well with those obtained by GC analysis of fatty acids.

Also, in the second region, the EVOO showed two peaks at 130.177 and 130.280 ppm assigned to C13 (DAG, TAG) (LO), C9 (DAG, TAG) (LN) which agreed with the GC analysis of fatty acids where EVOO had the highest content of linolenic acid (LN) (0.797 %) among other oils under investigation and had a considerable linoleic acid (LO) (11.043%). The fatty acids that need to be considered in EVOO are those based on C16 and C18 saturated alkanes (mainly palmitic and stearic acids) and C18 alkenes, monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) (mostly oleic, linoleic and linolenic acids). Olefinic, methylenic and carbonyl resonances were found to be suitable for the direct and structure-specific analysis of the relative amounts of single fatty acids present in EVOO.

However, MO had no peak at 128.170 ppm assigned to C10 (DAG, TAG) LO as present in the other three oils (EVOO, AKO and SO). This fall in line with the GC analysis of fatty acids where MO had the lowest content of linoleic acid (LO) (0.749%) which was much lower than other oils in the study.

The signals of glycerol backbone carbons appeared in the third region between 60 and 72 ppm revealed that all the oils had similar peaks in this region but with some differences in the values corresponding to their chemical shifts. MO had the least chemical shift of 62.167 ppm assigned to C1′ (TAG) followed by EVOO (62.175 ppm), then AKO and SO with the same value of 62.178 ppm. This may reflect the order of oils stability that it was as in the same array of the common Rancimat method where MO is the oil of the highest stability followed by EVOO then AKO and SO.

Also, there were shifts from other values assigned to C2′ (TAG), where, MO had the least chemical shift of 69.085 ppm, followed by SO (69.093 ppm) then AKO (69.097 ppm) and EVOO (69.104). These values may reflect only the stability of MO as the oil of the highest stability with the least values of chemical shifts in the glycerol backbone carbons region.

In the fourth region from 10 to 35 ppm that comprises signals of aliphatic carbons, it was noticed the presence of two peaks at 20.517 and 26.999 ppm in MO only assigned to C17 (all acylglycerols) LN; C14 (MAG, DAG, TAG) (LO). Three peaks were existed at 22.650, 25.572 and 25.856 ppm in SO only assigned to C17 (all acylglycerols) OL, SFA; C14 (all acylglycerols) LN; C11 (all acylglycerols) OL, LO, LN, respectively. Also, SO showed LO chains in both ^{1}H-NMR and ^{13}C-NMR more than EVOO, MO and AKO that agreed with GC analysis of fatty acids of oils.

When combining the results from ^{1}H-NMR, it was observed the absence of diacylglycerols in EVOO which mainly arise either from incomplete triacylglycerol biosynthesis or from limited lipase action on triacylglycerols.

5- SUMMARY

This study was planned to evaluate the stability of four extracted edible oils namely extra virgin olive oil (EVOO), moringa oil (MO), apricot kernel oil (AKO) and sunflower oil (SO). The oils were analyzed in a new manner by applying modern non-traditional instrumental methods of analysis, along with traditional ones as a reference to test the potency of the new analytical methods, in order to identify the relation between their composition and stability, with great stress to investigate what components are responsible to stability.

The oils were exposed to some familiar physical and chemical determinations such as color and refractive indices; acidity; saponification value; unsaponifiable matter %; iodine and peroxide values; ultraviolet absorbencies at 232, 270, 266, 274 nm; chlorophyll and carotenoids; as well as, the oxidative stability by Rancimat method and the antioxidant activity of phenolic extracts of oils by DPPH method.

The analytical methods extended to more sophisticated instrumental methods of analysis such as chromatographic separation and determination techniques involved gas chromatography (GC) with flam ionization detector in order to investigate the composition of fatty acids and sterols content of the oils. High performance liquid chromatography (HPLC) was also used to investigate the composition of polyphenols, flavonoids and tocopherols which proved their main causes of oils stability.

Fourier transform infra red (FTIR) and nuclear magnetic resonance (NMR) spectroscopic determinations technique were employed and applied as a potent, nondestructive and effective analytical tools to investigate the oxidative stability of the oils. FTIR spectroscopy determined the functional groups with their relative transmittances according to their concentrations in samples and their characteristic fingerprints. It also, determined the attributes of some functional groups to stability and monitoring the oxidation processes by following the changes in the spectra upon oxidation.

NMR investigate the differences between the tested oils to evaluate their oxidative stability

^{1}H-NMR helped in the identification of the protons of the main components of oils to show the difference in oils structures along with ^{13}C-NMR that helped in the identification of carbon skeleton of oils and their main components of carbonyl, olefinic, glyceryl and aliphatic carbons.

The obtained results are summarized as follows:

1- Physical and chemical characteristics of oils:

- The refractive index (RI) of oils increased in auto-oxidation and can be used to evaluate oils rancidity. The order of the obtained increasing RI values was in relation to linoleic acid (LO) content in tested oils except of EVOO due to the differences in structure between oils. MO had the least RI (1.4621) with the highest stability, followed by AKO (1.4647), then EVOO (1.4696), and at least SO having the highest RI (1.4739). Corresponding to 0.75, 23.13, 11.04 and 52.20% of LO, respectively.
- MO showed the highest red color value (3.6) followed by EVOO (2.2) then AKO (0.6) and SO (0.2) which correlated with the carotenoids content, where MO had the highest value followed by EVOO then AKO and SO, being 4.81, 3.21, 0.16 and 0.05 mg/kg, respectively. The blue color appeared only for EVOO and MO, with the highest value (3.2) for EVOO due to its highest chlorophyll content (2.78 mg/kg) comparable to MO (0.54 mg/kg) which had a low blue color index (0.5).
- The acidity of the oils formed by hydrolysis and oxidation, as well as, the peroxide value was too small to affect the quality of the tested oils.

- MO showed slightly higher saponification value (194.70) which reflect the short acyl chain of fatty acids, followed by AKO (193.50) then EVOO (186.10) and at least SO (183.00).
- EVOO characterized by the highest percentage of unsaponifiable matter (2.19%), followed by MO (1.90%), AKO (1.80%) then SO (1.36%), indicating the higher stability of EVOO and MO than others.
- The highest IV (116.96) was recorded for SO that was high unsaturated followed by AKO (105.37), then EVOO (81.46), and at least MO (73.65). The lower IV, the higher the resistance of oil oxidation, thus this value ordered the oils according to their stability as follows: MO > EVOO > AKO > SO.
- MO had the lowest absorption band at 232 nm followed by that of EVOO, then AKO, indicating the highest antioxidant effectiveness and the lower concentration of linoleate. However, SO gave rise to this intensity having relatively the highest value due to conjugated dienoic acid in this oil and its higher polyunsaturation. Thus, this UV absorption band arranged the oils according to their stability as: MO > EVOO > AKO > SO. The UV-absorption at 266 nm of EVOO was very small value, while for other oils were relatively higher. Nevertheless, all the measured wavelengths for all the tested oils were considered as small values.
- It was noticed that the relative ratios of PUFA/MUFA, $C_{16:0}/C_{18:2}$, $C_{18:1}/C_{18:2}$, $C_{18:2}/C_{18:3}$ and $[C_{18:2} + C_{18:3} / C_{18:1}]$ were correlated with the stability and ordered the tested oils as MO > EVOO > AKO > SO according to Rancimat method.
- EVOO had the highest content of chlorophyll followed by MO then SO and AKO. While, MO had the highest concentration of carotenoids followed by EVOO then AKO and SO, which reflect their stability and correlated positively with the oils oxidative stability measured by Rancimat, where, MO > EVOO > AKO > SO.

- EVOO had the highest total tocopherols and polyphenols followed by MO then AKO and SO, which arranged the oils descendingly according to their stability according to the order obtained by chlorophyll compounds.
- MO had the highest induction period (IP) being 190.00 hr indicating superior resistance to oxidation, followed by EVOO (59.11 hr) then AKO (26.60 hr). However, SO recorded only 7.45 hr.
- The highest resistance of MO to oxidation followed by EVOO was due to their less linoleate, higher percentage of oleate and unsaponifiable matter and the antioxidant content of tocopherols, flavonoids, sterols, phenolics, chlorophyll and carotenoids.
- The high stability of EVOO is mainly due to its relatively low degree of PUFA and its antioxidant activity of the unsaponifiable components, tocopherols, flavonoids and phenolic compounds which also showed synergistic effect with each other.
- AKO had a middle IP having relatively high stability due to its higher percentage of OL, but had higher LO and lower antioxidant content compared to MO and EVOO.
- The lowest IP of SO was due to its higher PUFA and lower antioxidant content and the higher degree of unsaturation.
- It was observed a decrease in the IP of the oils after removing their polyphenols that reflect the antioxidant activity of these compounds in the oils. It could be also observed that polyphenols were a main antioxidant in EVOO which content the highest value among other oils, and approached half of its IP. However, MO which had the highest stability included other factors other than polyphenols.
- The phenolic extract from EVOO had the highest value of radical scavenging capacity (92.91%) followed by MO (48.82%), AKO (45.18%) then SO (25.18).
- The highest percentage of β-sitosterol was found in EVOO followed by AKO, SO then MO. β-sitosterol besides

Δ^5-avenasterol may be mainly responsible for olive oil stability. It seems to be also a main factor in AKO and SO oxidative stability. MO contained the highest percentages of stigmasterol, campesterol and Δ^5-avenasterol among other oils which may be in totally responsible for its oxidative stability.

- The most abundant tocopherol in both EVOO and SO was α-tocopherol; and in AKO was γ-tocopherol, whereas, in MO was δ-tocopherol which its antioxidant activity exceeds that of α- and γ-tocopherol, and was contribution to the highest resistance to oxidation, and making clear the long IP of this oil.

2- Evaluation of oils stability using FTIR spectroscopy

- All the fresh studied oils showed many similarities between their FTIR spectral data. However, some variations were found, which successfully led to the differentiation between oils.
- The observed shifts of absorption peaks around 3005 cm^{-1} ordered the oils where the lowest shift of the peak was related to the highest stability as follows: MO (3003.59 cm^{-1}) > EVOO (3004.55 cm^{-1}) > AKO (3005.52 cm^{-1}) > SO (3006.48 cm^{-1}). This arrangement reflect the same order of oils stability evaluated by Rancimat method, and also correlated to the PUFA content of corresponding oils.
- The oil with lowest value of PUFA will have the lowest shift of absorption peak around 3005 cm^{-1} and will show the highest stability. In the same way, the PUFA arranged these oils as: MO (0.96%) > EVOO (11.84%) > AKO (23.56%) > SO (52.55%).
- Also, the higher in intensity of this peak around 3005 cm^{-1}, the highest in unsaturated fatty acids. From this relation, it was easy to predict the order and the appropriate values of the unsaturated fatty acids of edible oils.

- When the oil shows a peak around 3470 cm^{-1}, it is an indication that the oil is non-oxidized.
- MO which was evaluated as an oil of the highest stability by the Rancimat method, showed the lowest shifts of absorption peaks around 1236, 1162 and 1118 cm^{-1} followed by EVOO, then AKO and SO.
- The oxidized olive oil (Ox-OO) showed the similar peak at 1236 cm^{-1} with much higher intensity than the double of fresh EVOO.

3- Evaluation of oils stability using NMR spectroscopy:

3.1- ^{1}H-NMR spectral data analysis of fresh oils:

- In addition to main components, the studied oils contained minor components which are important because they show antioxidant stability. These components were present in high enough concentration to be detected by ^{1}H-NMR, and the signals of their protons did not overlap with those of the main lipid components.
- The chemical shifts of solvent peaks around 2.5 and 3.3 ppm ordered the oils stability, where the lesser shift was corresponded to the oil of higher stability. Accordingly, the order of oils stability obtained as follows: MO > EVOO > AKO > SO, agreed with the order of obtained by Rancimat method and with the order of tocopherols concentration as powerful natural antioxidants present in the oils.
- The higher oxidative stability of MO and EVOO than other oils is mainly due to their relatively low degree of fatty acid unsaturation and the antioxidant activity of some of the unsaponifiable components such as sterols. MO showed the presence of both stigmasterol and β-sitosterol from the signals at 0.662 and 0.784 ppm, respectively. However, EVOO showed a peak at 0.780 ppm assigned to β-sitosterol which is an important marker in olive oil analysis.

- The higher oxidative stability of MO over EVOO could be attributed to their fatty acid composition, both had higher OL content, EVOO contained LO and LN which were more readily undergoes oxidation and degradation than OL. These acids were in much lower quantities in MO. The higher stability was also due to other constituents of non-glyceride fraction that possess antioxidant properties. In addition, MO was the most rich in aliphatic chains.

3.2- ^{1}H-NMR spectral data analysis of stored oils after 6 months:

- The same peaks in fresh EVOO were still present in the stored oil. The slow and slight changes in oil stability caused some shifts in chemical assignments of ^{1}H-NMR signals.
- Additional peaks were appeared in ^{1}H-NMR spectrum of EVOO after 6 months. The most important peak was at 2.255 ppm attributed to free fatty acids due the hydrolytic degradation of triglycerides, thereby increasing the acidity of the oil.
- It was observed some differences between ^{1}H-NMR spectra of fresh MO, AKO and SO and their oils after storage for 6 months including the disappearance of some peaks and appearance of new peaks; also, some shifts were denoted in the spectra of the stored oils, attributed to free fatty acids and indicating the effect of hydrolytic degradation of acyl groups and the formation of new compounds, as well as conjugated systems.

3.3- ^{1}H-NMR spectral data analysis of EVOO after 60 months:

- Some chemical shifts of the ^{1}H-NMR signals of some compounds appeared after 6 months were still unmoved after 60 months, such as intensity of β-sitosterol signal.
- The ^{1}H-NMR spectrum of stored oil showed slight changes in intensity of triglycerides and of other components to lower intensities compared to fresh oil.

- The appearance of new signals in oxidized oil stored for 60 months were aroused due to the different kinds of alcohols, the free fatty acids and diglycerides resulted from the oxidation and degradation of the primary oxidative products. These compounds having protons whose signals in the ^{1}H-NMR spectrum did not overlap with any other, making it possible to identify.
- Combining the results of FA analysis and the oxidative stability of oils by Rancimat method, in general, the lower the content in polyunsaturated groups in the oil, the higher its resistance to degradation, however, other factors, such as the presence of minor antioxidant components, play an important role in degradation role of the oil.
- The ^{1}H-NMR spectroscopy is a very useful technique in the study of several aspects of edible oils and fats. It has the advantages that it is not destructive, requires a very small sample whose preparation is very simple and it takes little time.

3.4- ^{13}C-NMR spectral data analysis of fresh oils:

- No data were obtained from the ^{13}C-NMR spectroscopy of EVOO and SO dissolved in DMSO-d6. However, the results obtained from ^{13}C-NMR spectra of MO and AKO were very poor in data; the DMSO-d6 solvent occupied already all the spectra except a very few peaks appeared in aliphatic and glycerol regions.
- Thus, the use of DMSd6 was not suited as a solvent for the ^{13}C-NMR analysis of the studied oils. Another trial was carried out. When using the deuterated chloroform as a solvent, a large number of signals were spread over a wide range of chemical shifts.
- The sole $CDCl_3$ as a solvent showed a triplet chemical shifts around 77 ppm.

- Four spectral regions were observed in ^{13}C-NMR spectra that belong to four different groups of carbon resonances. The first one contained the carbonyl resonances of fatty acids; the second region involved resonances of the olefinic carbons of the unsaturated fatty acids; in the third region, the signals of glycerol backbone carbons appeared; and the fourth one comprised signals of aliphatic carbons.
- MO had the lowest chemical shifts of solvent peaks which reflect the highest stability of this oil among all other studied oils.
- In the first region, ranging from 172-174 ppm, it was observed the absence of peak resonating at 173 ppm characteristic of saturates (S) in AKO and SO, where it appeared in both EVOO and MO at 173.303 and 173.277 ppm, respectively. These finding agreed with the GC analysis of fatty acids.
- In the second region, ranging from 124-134 ppm, two peaks at 127.858 and 128.018 ppm assigned to LO were shown only in SO ^{13}C-NMr spectrum, that agreed with the GC analysis of FA, where, linoleic acid was the major fatty acid in SO (52.20%).
- Also, the absence of two peaks at 129.650 and 129.790 ppm in SO, while they present in other oils indicating that they had higher content of OL and lower content of LO than SO. EVOO showed also two peaks at 130.177 and 130.280 ppm indicating its highest content in LN and considerable LO among other oils. However, MO had no peak at 128.173 ppm as three other oils indicating the lowest content of LO.
- The signals of glycerol backbone carbons appeared in the third region between 60 and 72 ppm showed that all the oils had similar peaks with differences in values corresponding to their shifts. MO had the least chemical shifts followed by EVOO, then AKO and SO, this reflects the order of oils stability that it was the same obtained by Rancimat method.

- In the fourth region, from 10 to 35 ppm that comprises signals of aliphatic carbons, SO showed LO chains more than EVOO, MO and AKO that agreed with GC analysis of fatty acids of oils.
- ^{13}C-NMR technique is particularly useful in distinguishing between mono-, di- and tri-glycerides, as a strong discriminating power to detect the specific components and free fatty acids.
- When combining the results from ^{1}H-NMR it was observed the absence of diacylglycerols in EVOO which mainly arise either from incomplete triacylglycerol biosynthesis or from limited lipase action on triacylglycerols.

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الملخص العربي

تهدف هذه الدراسة إلى تقييم ثبات أربعة من الزيوت الغذائية مختلفة الثبات وهي زيت الزيتون البكر الممتاز، زيت المورينجا وزيت نوى المشمس (وهي زيوت مرتفعة الثبات) وزيت دوار الشمس (زيت منخفض الثبات). حيث تم إجراء التحاليل لدراسة خواص هذه الزيوت بطرق حديثة غير تقليدية باستخدام أجهزة التحليل الدقيق مع الطرق التقليدية المعروفة كطرق مرجعية لتقدير الثبات واستنباط العلاقة بين التركيب والثبات للزيوت. وكان الغرض أيضاً اختبار طرق تحليل حديثة ومعرفة مدى قدرتها في تقييم الثبات مع التركيز على معرفة المركبات المسئولة عن هذا الثبات.

ولقد تم إجراء بعض التحاليل التقليدية الطبيعية والكيميائية للزيوت مثل تقدير معاملات اللون والإنكسار، الحموضة، رقم التصبن، النسبة المئوية للمواد غير المتصبنة، الرقم اليودي، رقم البيروكسيد، إمتصاص الأشعة فوق البنفسجية عند الأطوال الموجية 232, 270, 266, 274 نانومتر، نسبة الكلوروفيل والكاروتينات، وكذلك تقدير الثبات الأكسيدي بطريقة الرانسيمات وتقدير النشاط المضاد للأكسدة للمستخلصات الفينولية للزيوت بطريقة DPPH.

ولقد تم التوسع في طرق التحليل للطرق الأكثر تعقيداً باستخدام أجهزة التحليل الدقيق المتقدمة وذلك بتحليل الزيوت بجهاز الفصل الكروماتوجرافي الغازى (GC/FID) لفصل وتقدير تركيب الأحماض الدهنية والإستيرولات. كذلك استخدم جهاز التحليل الكروماتوجرافي عالي الكفاءة (HPLC) لفصل وتقدير الفينولات المتعددة، الفلافونويدات والتوكوفيرولات والتي أثبتت ارتباطها بشكل كبير بالثبات العالي للزيوت عالية الثبات.

ولقد تم إجراء التحليل الطيفي للأشعة تحت الحمراء (FTIR) والرنين النووي المغناطيسي للبروتون (^{1}H-NMR) ونظير الكربون (^{13}C-NMR) والتي أثبتت فعاليتها كطرق سريعة لتقدير الثبات الأكسيدي للزيوت بدون التأثير على التركيب الخاص بالزيوت واستهلكت وقت قصير نسبياً بالمقارنة بالطرق التقليدية.

واستخدم التحليل الطيفي للأشعة تحت الحمراء (FTIR) للتعرف على المجموعات الفعالة مع تقدير شدة انبعاث الأشعة لكل منها تبعاً لتركيزات هذه المجموعات بالزيوت المختلفة والتي اعتبرت كبصمة الإصبع لكل زيت؛ أمكن استخدام هذه الخواص لتفرقة الزيوت عن بعضها البعض وكذلك معرفة علاقة بعض المجموعات الفعَّالة بالثبات مع متابعة التغيرات المصاحبة لعمليات الأكسدة عن طريق متابعة التغير في الطيف بتزايد معدل الأكسدة.

واستخدم التحليل الطيفي للرنين النووي المغناطيسي للبروتون (^{1}H-NMR) ونظير الكربون (^{13}C-NMR) للتعرف على الفرق في التركيب بين الزيوت المختبرة وعلاقتها بالثبات الأكسيدي. حيث ساعد التحليل الطيفي للرنين النووي المغناطيسي للبروتون (^{1}H-NMR) في التعرف على نوعية البروتونات بالمكونات الأساسية للزيوت واستخدامها لتفرقة الزيوت عن بعضها البعض. وساعد التحليل الطيفي للرنين المغناطيسي لنظير الكربون (^{13}C-NMR) في التعرف على الهيكل الكربوني للزيوت وكذلك مكوناتها الأساسية من ذرات كربون كربونيلية، أوليفينية، جلسرولية وأليفاتية.

وفيما يلي ملخص لأهم النتائج المتحصل عليها:

1- الخواص الطبيعية والكيميائية للزيوت:

- سجل معامل الإنكسار للزيوت زيادة في قيمته بزيادة الأكسدة التلقائية وأمكن استخدامه لتقدير تزنخ وفساد الزيوت. وكان ترتيب قيم معاملات الإنكسار مرتبط بمحتوى حامض اللينوليك (LO) بالزيوت محل الإختبار باستثناء زيت الزيتون البكر الممتاز ويرجع ذلك للفروق في التركيب. وسجَّل زيت المورينجا أقل قيمة لمعامل الإنكسار (1.4621) مع أعلى ثبات، يليه زيت نوى المشمش (1.4647) ثم زيت الزيتون البكر الممتاز (1.4696) وجاء زيت دوار الشمس في المرتبة الأخيرة مسجلاً أعلى قيمة لمعامل الإنكسار (1.4739) وكان ذلك مناظراً لقيم تركيزات حامض اللينوليك (LO) التي بلغت 0.75، 23.13، 11.04، 52.20% للزيوت السابقة على الترتيب.
- أظهر زيت المورينجا أعلى قيمة لمعامل اللون الأحمر (3.6)، يليه زيت الزيتون البكر الممتاز (2.2) ثم زيت نوى المشمش (0.6) وزيت دوار الشمس (0.2) والذي ارتبط بمحتوى الكاروتينات بالزيت، حيث احتوى زيت المورينجا على أعلى محتوى منها، يليه زيت الزيتون البكر الممتاز ثم زيت نوى المشمش وزيت دوار الشمس في المرتبة الأخيرة. وكانت قيم الكاروتينات للزيوت السابقة 4.81، 3.21، 0.16، 0.05 مللجم/كجم، على الترتيب. ولقد ظهر معامل اللون الأزرق فقط بزيت الزيتون البكر الممتاز وزيت المورينجا مسجلاً أعلى قيمة له (3.2) لزيت الزيتون البكر الممتاز نتيجة لإحتواءه على أعلى نسبة من الكلوروفيل (2.78 مللجم/كجم) بالمقارنة بزيت المورينجا (0.54 مللجم/كجم) الذي له معامل للون الأزرق له 0.5.

- وكانت القيم المسجَّلة للحموضة ورقم البيروكسيد للزيوت غير ملحوظة بدرجة تجلعها **لا** تؤثر في جودة الزيوت محل الدراسة.
- وأظهر زيت المورينجا قيمة أعلى بنسبة بسيطة لرقم التصبن (194.70) عن الزيوت الأخرى والذي يعكس وجود سلسلة آسايل قصيرة للأحماض الدهنية، يليه زيت نوى المشمش (193.50) ثم زيت الزيتون البكر الممتاز (186.10) **وكان** زيت دوار الشمس (183.00) في المرتبة الأخيرة.
- ولقد كانت نسبة المواد غير المتصبنة مميزة جداً لزيت الزيتون البكر الممتاز حيث **سجَّل** أعلى قيمة لها (2.19%) يليها زيت المورينجا (1.90%) **ثم** زيت نوى المشمش (1.80%) وزيت عباد الشمس (1.36%) **الذي كان في** المرتبة الأخيرة، **مما** يدل على الثبات الأعلى لزيت الزيتون البكر الممتاز وزيت المورينجا.
- **وكان** أعلى رقم يودي مسجَّلاً لزيت دوار الشمس (119.96) الذي به نسبة عالية من عدم التشبع، يليه زيت **نوى المشمش** (105.37) ثم زيت الزيتون البكر **الممتاز** (81.46) وزيت المورينجا (73.65) في المرتبة الأخيرة. ولوحظ أنه كلما قل الرقم اليودي زادت مقاومة الزيت للأكسدة وبالتالي فإنه تبعاً للرقم اليودي فقد تم ترتيب الزيوت تبعاً لدرجة ثباتها كما يلي: زيت المورينجا > زيت الزيتون البكر **الممتاز** > زيت نوى المشمش > زيت دوار الشمس.
- وأظهر زيت المورينجا أقل إمتصاص بالطول الموجي 232 **نانومتر** يليه زيت الزيتون البكر الممتاز ثم زيت نوى المشمش وذلك يدل على وجود أعلى **نشاط** مضاد للأكسدة مع أقل تركيز من اللينوليبات (LO). **وعلى الرغم من ذلك** فإن زيت دوار الشمس سجَّل أعلى قيمة لشدة الإمتصاص نتيجة لوجود حامض الدايينويك المقترن بهذا الزيت مع النسبة الأعلى من تعدد عدم التشبُّع. **وبالتالي، فإن** تحليل طيف الإمتصاص بالمنطقة فوق البنفسجية جعل ترتيب الزيوت تبعاً لثباتها كما يلي: زيت المورينجا > زيت الزيتون البكر الممتاز > زيت نوى المشمش > زيت دوار الشمس. وكان مقدار الإمتصاص للأشعة فوق البنفسجية بالطول الموجي 266 نانومتر لزيت الزيتون البكر الممتاز صغيراً جداً وكان أعلى نسبياً لباقي الزيوت. **وعلاوة على ذلك فإن** هذه القياسات التي أجريت للزيوت كلها كانت قيمها كلها صغيرة.
- ولقد لوحظ ارتباط نسب رياضية مستنتجة من تركيب الأحماض الدهنية **بالثبات**، حيث كانت قيم **النسب**: PUFA/MUFA ، $C_{16:0}/C_{18:2}$ ، $C_{18:1}/C_{18:2}$ ، $C_{18:2}/C_{18:3}$ ، $[C_{18:2} + C_{18:3} / C_{18:1}]$ متوافقة مع ترتيب الثبات للزيوت كما

يلي: زيت المورينجا > زيت الزيتون البكر الممتاز > زيت نوى المشمش > زيت دوار الشمس.

- ولقد احتوى زيت الزيتون البكر الممتاز على أعلى نسبة من الكلوروفيل يليه زيت المورينجا ثم زيت دوار الشمس، وزيت نوى المشمش كان في المرتبة الأخيرة. بينما احتوى زيت المورينجا على أعلى نسبة من الكاروتينات يليه زيت الزيتون البكر الممتاز ثم زيت نوى المشمش وزيت دوار الشمس كان في المرتبة الأخيرة. وذلك يعكس ثبات الزيوت الذي يتوافق ترتيبه مع نفس ترتيب الثبات المقدر بطريقة الرانسيمات حيث كان زيت المورينجا > زيت الزيتون البكر الممتاز > زيت نوى المشمش > زيت دوار الشمس.

- احتوى زيت الزيتون البكر الممتاز على أعلى نسبة من التوكوفيرولات الكلية والفينولات المتعددة، يليه زيت المورينجا ثم زيت نوى المشمش وزيت دوار الشمس في المرتبة الأخيرة. وكان ترتيب الزيوت وفقاً للثبات كما في ترتيبهم وفقاً لمحتوى المركبات الكلوروفيلية مماثلاً لترتيبهم للثبات بالرانسيمات.

- وسجل زيت المورينجا أعلى قيمة للفترة التحضيرية (IP) والتي وصلت إلى 190ساعة والذي كان الزيت الأكثر مقاومة للأكسدة، يليه زيت الزيتون البكر الممتاز (59.11ساعة) ثم زيت نوى المشمش (26.60ساعة)، وبعد ذلك سجل زيت دوار الشمس الذي سجل فقط 7.45ساعة.

- وكانت أعلى مقاومة للأكسدة مسجَّلة لزيت المورينجا يليها زيت الزيتون البكر الممتاز نتيجة لمحتواهم القليل من اللينوليات (LO) مع النسبة الأعلى للأولييات (OL) والمواد غير المتصبنة ومحتوى مضادات الأكسدة من الفلافونويدات، الإستيرولات، الفينولات، الكلوروفيل والكاروتينات.

- ويرجع الثبات العالي لزيت الزيتون البكر الممتاز إلى محتواه المنخفض من الأحماض الدهنية متعددة عدم التشبع (PUFA) والنشاط المضاد للأكسدة للمواد غير المتصبنة، التوكوفيرولات، الفلافونويدات والمركبات الفينولية والتي أظهرت تأثير تعاوني ككل أقوى من تأثير المكونات المنفردة.

- وسجَّل زيت نوى المشمش قيمة متوسطة للمرحلة التحضيرية (IP) وكان له ثبات عالي نسبياً نتيجة احتواءه على نسبة عالية من حامض الأولييك (OL) ولكنه احتوى على نسبة أعلى من اللينولييك (LO) ومحتواه قليل من مضادات الأكسدة بالمقارنة بزيت المورينجا وزيت الزيتون البكر الممتاز.

- وكانت أقل قيمة للمرحلة التحضيرية (IP) لزيت دوار الشمس نتيجة لمحتواه الأعلى من الأحماض الدهنية متعددة عدم التشبع (PUFA) ومحتواه القليل من مضادات الأكسدة وله درجة أعلى في عدم التشبع.

- ولقد لوحظ وجود إنخفاض في قيمة الفترة التحضيرية (IP) للزيوت بعد نزع الفينولات المتعددة منها وذلك يعكس دور النشاط المضاد للأكسدة للفينولات المتعددة بالزيوت. وقد أمكن ملاحظة أن الفينولات المتعددة كانت تمثل مضادات الأكسدة الرئيسية بزيت الزيتون البكر الممتاز وهي الأعلي فيه بالمقارنة بباقي الزيوت محل الدراسة واقتربت قيمة الفترة التحضيرية (IP) من نصف قيمتها قبل نزع الفينولات. وبالرغم من ذلك فإن زيت المورينجا أظهر أعلى ثبات مما يدل على وجود عوامل أخرى لزيادة الثبات غير الفينولات المتعددة.
- سجَّل المستخلص الفينولي لزيت الزيتون البكر الممتاز أعلى قيمة للقدرة على اصطياد الشقوق الحرة (%92.91) يليه زيت المورينجا (%48.82) ثم زيت نوى المشمش (%45.18) وزيت دوار الشمس (%25.18) كان في المرتبة الأخيرة.
- كانت أعلى نسبة من بيتا-سيتوستيرول موجودة بزيت الزيتون البكر الممتاز، يليه زيت نوى المشمش ثم زيت دوار الشمس وزيت المورينجا كان في المرتبة الأخيرة. وقد يكون وجود بيتا-سيتوستيرول مع Δ^5-أفيناستيرول هو سبب رئيسي للثبات العالي لزيت الزيتون البكر الممتاز. ويبدو أنه أيضاً عامل رئيسي في الثبات الأكسيدي لزيت نوى المشمش وزيت دوار الشمس. واحتوى زيت المورينجا على أعلى نسب من ستيجماستيرول، كامبيستيرول و Δ^5-أفيناستيرول بالمقارنة بباقي الزيوت والتي اعتبرت ككل مسئولة عن الثبات الأكسيدي لزيت المورينجا.
- كان ألفا-توكوفيرول هو الأكثر محتوى في كل من زيت الزيتون البكر الممتاز وزيت دوار الشمس؛ وكان جاما-توكوفيرول هو الأكثر محتوى في زيت نوى المشمش. بينما كان دلتا-توكوفيرول هو الأكثر محتوى بزيت المورينجا والذي ظهر له نشاط مضاد للأكسدة أعلى من ألفا- وجاما-توكوفيرول، وهو سبب رئيسي للمقاومة الأعلى للأكسدة وارتفاع قيمة الفترة التحضيرية (IP).

2- تقدير ثبات الزيوت بطريقة التحليل الطيفي للأشعة تحت الحمراء:

- أظهرت كل الزيوت غير المخزنة عديد من التشابهات في نتائج التحليل الطيفي للأشعة تحت الحمراء لها. وبالرغم من ذلك، فقد وجدت بعض الإختلافات التي أدت للتفرقة بين الزيوت محل الدراسة.
- ظهرت إزاحات لقمم الإمتصاص قرب 3005سم$^{-1}$ والتي رتبت الزيوت بحيث كانت الإزاحة الأقل مناظرة للثبات الأعلى كما يلي: زيت المورينجا (3003.59سم$^{-1}$) > زيت الزيتون البكر الممتاز (3004.55سم$^{-1}$) > زيت نوى

المشمش (3005.52 سم$^{-1}$) > زيت عباد الشمس (3006.48 سم$^{-1}$). ويتوافق الترتيب السابق مع نفس الترتيب بطريقة الرانسيمات وكذلك يرتبط بنسبة الأحماض الدهنية عديدة عدم التشبع (PUFA).

- كان الزيت صاحب أقل قيمة من الأحماض الدهنية عديدة عدم التشبع (PUFA) هو صاحب أقل إزاحة للقمة قرب 3005سم$^{-1}$ وذلك يبين الثبات العالي للزيت. وبنفس الطريقة فإن الأحماض الدهنية عديدة عدم التشبع (PUFA) رتبت هذه الزيوت كما يلي: زيت المورينجا (0.96%) > زيت الزيتون البكر الممتاز (11.84%) > زيت نوى المشمش (23.56%) > زيت دوار الشمس (52.55%).
- أيضاً، كلما زادت شدة الإمتصاص بهذه القمة قرب 3005 سم$^{-1}$، كلما كانت النسبة الأعلى من الأحماض الدهنية غير المشبعة (USFA). وقد أمكن استخدام هذه العلاقة للتوقع بالترتيب والقيم المناسبة للأحماض الدهنية غير المشبعة بالزيوت الغذائية.
- عندما يظهر الزيت قمة للإمتصاص قرب 3470سم$^{-1}$، فإن ذلك يدل على أن الزيت غير-مؤكسد.
- زيت المورينجا الذي تم تقييمه كأعلى زيت في الثبات بطريقة الرانسيمات التقليدية، أظهر أقل قيمة للإزاحة لقمم الإمتصاص قرب القمم 1236، 1162، 1118سم$^{-1}$، يليه زيت الزيتون البكر الممتاز ثم زيت نوى المشمش وزيت دوار الشمس في المرتبة الأخيرة.
- أظهر زيت الزيتون المؤكسد قمة إمتصاص مشابهة عند القيمة 1236سم$^{-1}$ وكانت شدتها أكبر من ضعف القيمة لزيت الزيتون البكر الممتاز غير المخزن مما ساعد على التفرقة بينهما.

3- تقدير ثبات الزيوت بطريقة التحليل الطيفي للرنين النووى المغناطيسي:

3.1- تحليل طيف الرنين النووى المغناطيسي للبروتون للزيوت غير المخزنة:

- علاوة على وجود المكونات الأساسية، فقد احتوت الزيوت محل الدراسة على مكونات صغري ذات أهمية حيث أظهرت ثبات أكسيدي. وكانت هذه المكونات موجودة بتركيز عالي بدرجة كافية يمكن الكشف عنها بتحليل طيف الرنين النووي المغناطيسي للبروتون، ولم يحدث تداخل لإشاراتها مع تلك الخاصة بالمكونات الأساسية لليبيدات.

- ظهرت قيم الإزاحات الكيميائية لقمم المذيب قرب 2.5 ، 3.3 **جزء فى** المليون وذلك أعطي ترتيب لثبات الزيوت بحيث كانت الإزاحة الأقل مناظرة للزيت الأعلى ثباتاً. ووفقاً لذلك، كان ترتيب الثبات للزيوت كما يلي: زيت المورينجا > زيت الزيتون البكر **الممتاز** > زيت نوى المشمش > زيت دوار الشمس، حيث اتفق هذا الترتيب مع ترتيب طريقة الرانسيمات وكذلك مع ترتيب تركيزات التوكوفيرولات كمضادات أكسدة طبيعية قوية بالزيوت.
- يرجع الثبات الأكسيدي الأعلي لزيت المورينجا وزيت الزيتون البكر الممتاز بالمقارنة بالزيوت الأخرى، إلى درجة عدم التشبع القليلة نسبياً للأحماض الدهنية وكذلك سبب وجود نشاط مضاد الأكسدة لبعض المكونات غير المتصبنة مثل الإستيرولات. وأظهر زيت المورينجا وجود كل من ستيجماستيرول وبيتا-سيتوستيرول وذلك من الإشارات عند 0.662 ، 0.748 جزء فى المليون، **على الترتيب**. وبرغم ذلك فإن زيت الزيتون البكر الممتاز أظهر قمة عند 0.780 **جزء** فى المليون مخصصة للبيتا-سيتوستيرول وهي علامة هامة في تحليل زيت الزيتون.
- ويرجع تفوق زيت المورينجا على زيت الزيتون البكر **الممتاز في الثبات** إلى تركيب الأحماض الدهنية؛ كلاهما يحتوي على نسبة عالية من الأولييك (OL)، زيت الزيتون البكر الممتاز يحتوى على اللينولييك (LO) واللينولينيك (LN) وهي سهلة التأكسد و**التحلل** بالمقارنة بحامض الأولييك (OL). وتوجد هذه الأحماض سهلة **التحلل** بتركيزات **أقل في** زيت المورينجا. ويرجع ثبات زيت المورينجا **الأعلى** أيضاً لوجود مكونات أخرى غير-جليسيرايدية لها خواص مضادة للأكسدة. وعلاوة على ذلك فإن زيت المورينجا هو الأكثر إحتوائاً على سلاسل أليفاتية.

3.2- تحليل طيف الرنين النووى المغناطيسي للبروتون للزيوت بعد التخزين لمدة 6 شهور:

- احتفظ زيت الزيتون البكر الممتاز بنفس الإشارات لقمم الطيف حتى بعد التخزين لمدة 6 شهور. ولقد تسببت التغيرات البطيئة الطفيفة في إحداث بعض الإزاحات لإشارات طيف الرنين النووي المغناطيسي للبروتون.
- ظهرت إشارات لقمم إضافية كان أهمها عند 2.255 جزء فى المليون **ترجع لوجود أحماض** دهنية حرة نتيجة للتحلل **المائي** للجليسريدات الثلاثية، وبالتالي زيادة حموضة الزيت.

- لوحظت بعض الإختلافات بين أطياف الرنين النووي المغناطيسي لزيوت المورينجا، نوى المشمش ودوار الشمس الغير مخزنة وبعد التخزين لمدة 6 شهور، اشتملت على اختفاء بعض إشارات القمم وظهور إشارات لقمم جديدة وظهرت بعض الإزاحات لقمم نتيجة لتكوين الأحماض الدهنية الحرَّة مما يدل على حدوث تحلل مائي لمجموعات الآسايل وتكوين مركبات جديدة مع أنظمة مقترنة.

3.3- تحليل طيف الرنين النووى المغناطيسي للبروتون لزيت الزيتون البكر الممتاز بعد التخزين لمدة 60 شهر:

- كانت بعض الإشارات لقمم أطياف الرنين النووي المغناطيسي للبروتون لبعض المركبات التي ظهرت بعد 6 شهور من التخزين لا تزال موجودة لن تتغير حتى بعد 60 شهر من التخزين، مثل بيتا-سيتوستيرول.
- أظهر طيف الرنين النووي المغناطيسي للبروتون للزيت بعد التخزين لمدة 60 شهر وجود تغيرات في شدة إشارات الجليسيرايدات الثلاثية بحيث إنخفضت بالمقارنة بالزيت غير المخزن.
- ظهرت إشارات جديدة في الزيت المؤكسد المخزن لمدة 60 شهر نتيجة لظهور أنواع مختلفة من الكحولات، الأحماض الدهنية الحرة والجليسيرايدات الثنائية الناتجة من التحلل ومن تفكك منتجات الأكسدة الأولية. وكانت هذه المركبات ظاهرة بوضوح وأمكن تمييزها بدون تداخل مع إشارات أخرى بالطيف.
- بعمل دمج لنتائج تحليل الأحماض الدهنية والثبات الأكسيدي بطريقة الرانسيمات التقليدية، فإنه بصورة عامة، كلما قل محتوى الأحماض الدهنية عديدة عدم التشبع (PUFA) بالزيت، كلما كانت مقاومته أعلى للأكسدة، وبرغم ذلك فهناك عوامل أخرى مثل وجود المكونات الصغرى كمضادات أكسدة والتي لعبت دوراً هاماً في حماية الزيت من الأكسدة.
- أثبتت طريقة تحليل طيف الرنين النووي المغناطيسي مقدرتها على دراسة مظاهر عديدة بالزيوت والدهون الغذائية. وتتميز هذه الطريقة بأنها تقوم بالتحليل للعينة بأقل كمية وفي وقت قصير نسبياً.

3.4- تحليل طيف الرنين النووى المغناطيسي لنظير الكربون (^{13}C-NMR):

- عند إذابة زيت الزيتون البكر الممتاز وزيت دوار الشمس في ثنائي ميثايل السلفوكسيد الديوتيري، لم تظهر بيانات واضحة بالتحليل الطيفي للرنين النووي

المغناطيسي لنظير الكربون. **وبرغم** ذلك، ظهرت نتائج لزيت المورينجا وزيت **نوى المشمش و**لكنها لم تكن مرضية حيث إحتل المذيب تقريباً معظم مناطق الطيف باستثناء إشارات قليلة لقمم خاصة بالمناطق الأليفاتية والجليسرولية بالطيف.

- ثبت عدم نجاح ثنائي ميثايل السلفوكسيد الديوتيري كمذيب في التحليل الطيفي للرنين النووي المغناطيسي لنظير الكربون للزيوت محل الدراسة. **ولذلك تم** إجراء تجربة أخرى بإستخدام مذيب الكلوروفورم الديوتيري والذي أثبت نجاحه وظهر عدد كبير من إشارات القمم بالتحليل الطيفي.
- أظهر الكلوروفورم الديوتيرى كمذيب للزيوت وجود ثلاثة إشارات لقمم قرب القيمة 77 جزء فى المليون.
- لوحظ وجود أربعة مناطق بطيف الرنين النووي المغناطيسي لنظير **الكربون مخصصة لأربعة مجموعات مختلفة من مركبات الكربون. كانت** المجموعة الأولى خاصة بمجموعة الكربونيل للأحماض الدهنية؛ المنطقة الثانية اشتملت على ذرات الكربون الأوليفينية للأحماض الدهنية غير المشبعة؛ المنطقة الثالثة بها إشارات ذرات كربون هيكل الجليسرول؛ والمنطقة الرابعة اشتملت على ذرات الكربون الأليفاتية.
- أظهر زيت المورينجا أقل إزاحة كيميائية لإشارات قمم المذيب وذلك يعكس ثباته **الأعلى بالم**قارنة بباقي الزيوت محل الدراسة.
- **بالمنطقة الأولي** (172-174 جزء فى المليون) لوحظ غياب إشارة مخصصة لقمة مميزة للمكونات المشبعة في زيت نوى المشمش وزيت دوار الشمس، حيث ظهرت في كل من زيت الزيتون البكر الممتاز وزيت المورينجا عند القيم 173.303، 173.277 **ج ف م. على** الترتيب. وتوافقت هذه النتائج مع التحليل الكروماتوجرافي للأحماض الدهنية بالزيوت.
- بالمنطقة الثانية (124-134 جزء فى المليون) ظهرت إشارتان عند 127.858، 128.018 جزء فى المليون مخصصة للينولييك (LO) وذلك بزيت دوار الشمس فقط والتي توافقت مع التحليل الكروماتوجرافي للأحماض الدهنية، وكان حامض اللينولييك (LO) هو الأكثر تركيزاً (52.20%) بزيت دوار الشمس.
- لوحظ أيضاً غياب إشارتين عند 129.650، 129.790 جزء فى المليون بزيت دوار الشمس وظهورهما بالزيوت الأخري لتدل على وجود المحتوى الأعلى من الأولييك (OL) **والمحتوي الأقل من** اللينولييك (LO) بالمقارنة بزيت دوار **للشمس**. وأظهر زيت الزيتون إشارتين عند 130.177، 130.280 **جزء فى** المليون مخصصة لمحتواه من اللينولينيك (LN) الذي يعتبر الأعلي ضمن مقارنته **بالزيوت الأخرى** مع وجود نسبة عالية من الأولييك (OL). **وجد أن** زيت

المورينجا لم تظهر له أية إشارات عند 128.173 جزء فى المليون مثل الزيوت الثلاثة السابقة وذلك لإحتواءه على أقل نسبة من اللينولييك (LO).

- ظهرت إشارات هيكل الجليسرول بالمنطقة الثالثة (60-72 **جزء فى** المليون) حيث أظهرت كل الزيوت نفس المجموعة من الإشارات مع إختلاف قيم الإزاحات الكيميائية لها. وكان زيت المورينجا الأقل إزاحة بين الزيوت يليه زيت الزيتون البكر الممتاز ثم زيت نوى المشمش وزيت دوار الشمس في المرتبة الأخيرة. ويعكس ذلك ترتيب الثبات للزيوت وهو نفس ترتيب طريقة الرانسيمات.

- واشتملت المنطقة الرابعة بالطيف (10-35 جزء فى المليون) **على ذرات** الكربون الأليفاتية، حيث أظهر زيت دوار الشمس وجود سلاسل اللينولييك (LO) أكثر من زيت الزيتون البكر الممتاز، زيت المورينجا وزيت نوى المشمش والذي يتوافق مع التحليل الكروماتوجرافي للأحماض الدهنية للزيوت محل الدراسة.

- أفاد تحليل طيف الرنين النووي المغناطيسي لنظير الكربون في التفرقة بين الجليسريدات الأحادية، الثنائية والثلاثية، وهو أداة قوية للكشف عن المكونات المتخصصة والأحماض الدهنية الحرة بالزيت.

- **بدمج ال**تحليل الطيفي للرنين النووي المغناطيسي للبروتون مع نظير ذرة الكربون، يلاحظ غياب مركبات ثنائي آسايل الجليسرول في زيت الزيتون البكر الممتاز والتي تظهر كنتيجة للتخليق الحيوي الغير مكتمل لثلاثي آسايل الجليسرول أو من تأثير إنزيم الليبيز المحدود على مركبات ثلاثي آسايل الجليسرول.

جامعة عين شمس
كلية الزراعة

رسالة دكتوراه

اسم الطالبة: **رانيا إبراهيم محمد المصيلحي**
عنوان الرسالة: اتجاهات جديدة في ثبات بعض الزيوت الغذائية
اسم الدرجة: **دكتور فلسفة في العلوم** الزراعية **(علوم وتكنولوجيا** الأغذية)

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صفحة الموافقة على الرسالة

اتجاهات جديدة في ثبات بعض الزيوت الغذائية

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